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EUNUCHOIDISM WITH LOW URINARY FOLLICLE-STIMULATING HORMONE IN THE FEMALE: COMPARISON WITH THIS SYNDROME IN THE MALE AND WITH THE PRE-MENARCHAL MENOPAUSE*†

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ABSTRACT

Four cases are presented of a syndrome in the female characterized by eunuchoidism without other endocrine abnormality and by an absence of urinary follicle-stimulating hormone (biologic assay). The syndrome is considered to be due to absence of pituitary FSH specifically, but it is possible that absence of pituitary luteinizing hormone is also a factor. The syndrome is contrasted with the pre-menarchal menopause, from which it is indistinguishable clinically, but in which there is a high titer of urinary FSH. It is also compared to eunuchoidism with low urinary FSH in the male, a syndrome with which it is etiologically identical. The results of treatment with

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human chorionic gonadotropin differ greatly, however, between males and females with this condition. In the male, administration of HCG may result in complete testicular development, but prolonged studies with HCG in the female have failed to demonstrate any effect.

SINCE the introduction of quantitative techniques for measuring urinary follicle-stimulating hormone (FSH) in our laboratory in 1942 (1), we have observed 13 women with normal stature in whom sexual development had failed to occur. Assay of urinary FSH permits division of these patients with eunuchoidism into two distinct groups: those with low FSH, and those with high FSH (2).

This paper is concerned primarily with 4 patients in whom eunuchoidism was due to an apparently selective absence or insufficiency of gonadotropins. The endocrine pattern in these women was similar to that normally present before puberty—the pituitary produces no measurable FSH and the ovary remains quiescent. Why the pituitary was inactive in respect to FSH production is not known; in none of the patients was there evidence of pituitary tumor or of other pituitary or hypothalamic disorder. The youngest of these women is now 28 years of age, so it is unlikely that any of these cases is an example of delayed puberty, from which eunuchoidism with low FSH must always be distinguished. In none was there evidence of an ovarian or adrenal tumor or of a debilitating disease which might suppress pituitary function.

EUNUCHOIDISM WITH LOW FSH IN THE FEMALE—DESCRIPTION OF 4 CASES

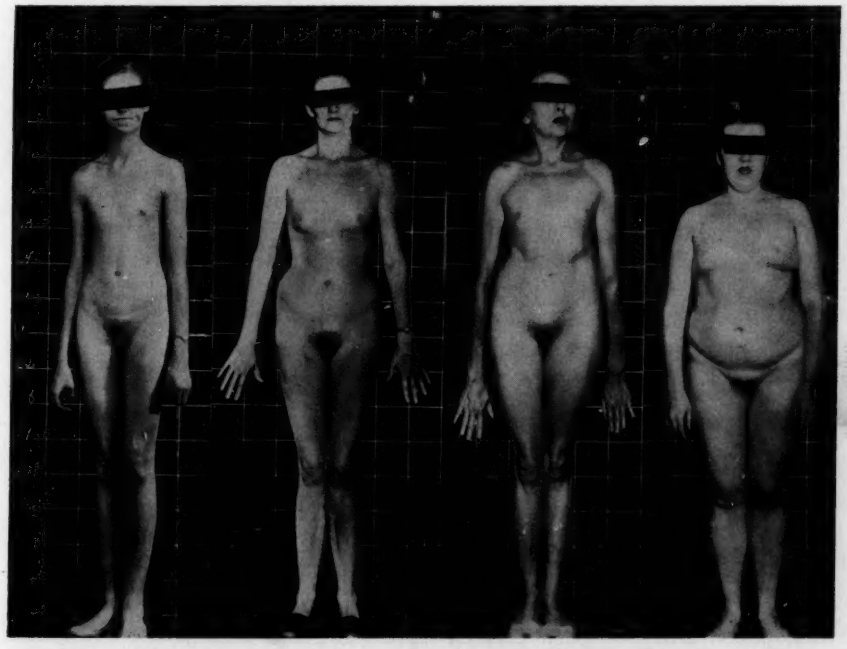
(see Appendix for clinical summaries)

The 4 women without measurable urinary FSH were all in good general health, with the single complaint of failure of sexual development. Their ages ranged from 18 to 41 years when they were first seen (Table 1 and Fig. 1). In none had there been development of the breasts prior to hormonal treatment, and none had ever menstruated. The hair on the head and extremities was not remarkable, but there was less than the normal adult amount of axillary and pubic hair. In all cases, span measurements were greater than those for height, which ranged from 62 inches to 70 inches. Examination of the genitalia in each revealed a small vagina and a small normal-appearing cervix. In the 2 patients in whom it was palpable, the uterus was small. The external genitalia were underdeveloped. No abnormal pelvic masses were felt. In 2 patients the ovaries were observed at operation; in both they were prepubertal in appearance. Biopsy in 1 patient showed essentially normal prepubertal ovarian tissue; the follicles were immature and may have been fewer than normal (Fig. 2). Examination of cells from the buccal mucosa of the 2 patients on whom laparotomy was performed revealed high percentages of "chromatin positive" nuclei, indicating female genetic sex (3).

TABLE 1. TYPES OF FSH EXCRETION IN FEMALE EUNUCHOIDS WITH NORMAL STATURE

Patient	Age (yrs.)		Height (inches)	Span (inches)	Weight (lbs.)	Urinary excretion		
	When first seen	When last seen				FSH (m.u./24 hrs.)	17-KS (mg./24 hrs.)	LH
Low FSH								
C.W. (#229892)	18	34	68½	68¾	103	< 3	6.1	
M.DiM. (#459716)	36	42	64	71	112	< 6.5	3.3	0
H.L. (#719481)	26	28	67½	69¼	128	< 6.5	6.8	
M.F. (#739966)	28	32	62	65¼	152	< 6.5	3.7	
High FSH								
M.G. (#181822)	33		68		122	> 10 m.u./100 cc.	5.1	
E.C. (#150632)	16	21	65½		142	>192	2.4	
A.B. (#361212)	34	39	60½		115	>192	7.8	
E.S. (#505730)	48	58	69		154	>104	5.4	
R.H. (#282915)	35		64¾		191	Qualitatively positive	8.4	
I.S. (#454504)	27	33	64		96	>384	1.4	
G.M.(PP*)	18	20	63		133	>192	3.6	
E.B. (PP*)	22	23	61		100	>384		
R.N. (#128962)	53	54	60½		148	>104		

* Private patient of F.A.



C. W. H. L. M. DiM M. F.

FIG. 1. Four female eunuchoids with low urinary FSH. Breast development in Patient H. L. was induced by a brief course of stilbestrol which she received before this photograph was taken.

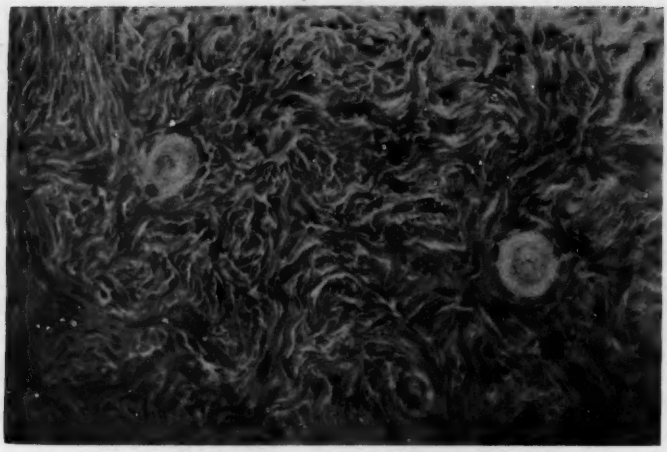


FIG. 2. Histologic section from ovary of Patient C. W., aged 22. The follicles are sparse and immature.

By history and physical examination no other endocrine defects were found. With the exception of negative assays for urinary FSH in all 4 patients, decreased 17-ketosteroid excretion in 2, and low-normal 17-ketosteroid excretion in 2, the results of endocrinologic laboratory studies were normal. By radiography of the skull and examination of the visual fields there was no evidence of pituitary neoplasm. Radiologic examination of the spine in each patient showed mild osteoporosis. Although some vertebrae appeared slightly wider in anterior-posterior diameter and more shallow in vertical diameter than normal, there was no epiphysitis.

COMPARISON WITH CASES OF EUNUCHOIDISM WITH HIGH FSH IN THE FEMALE ("PREMENARCHAL MENOPAUSE")

On the basis of the history and physical examination, the patients with low FSH could not be distinguished from those with high FSH (Table 1). Except for the FSH titers, the results of laboratory examinations in both groups were similar.

In the group of 9 patients with high levels of urinary FSH, three and possibly more subdivisions were theoretically possible:

1) The ovaries may be present, but they are defective and unresponsive to gonadotropins. This hormonal pattern is comparable to that of the menopause and has been termed "premenarchal menopause" (2). The ovary fails to respond and ovarian secretions are not present to inhibit pituitary secretion.

2) The ovaries may be absent or rudimentary. Such patients would be

similar to those with "gonadal dysgenesis," but without short stature and other stigmata of Turner's syndrome (4–7).

3) The ovaries may be normal but the pituitary produces only FSH—without luteinizing hormone (LH or ICSH) and possibly without luteotropic hormone (LTH). One might then expect follicular enlargement, but no production of estrogen. As we did not perform assays for LH or LTH, it is not known whether this hypothetical group describes any of these patients.

None of the 9 eunuchoid patients with high FSH had known ovarian inflammation or had undergone radiation or surgical treatment of the ovaries. Any of these, occurring before puberty, may lead to eunuchoidism with high FSH.

COMPARISON WITH CASES OF EUNUCHOIDISM WITH LOW FSH IN THE MALE

What we believe to be the male counterpart of the syndrome under discussion has been previously described (8–11). Whereas eunuchoidism with low FSH is rare in females, it is the most common type of eunuchoidism in the male. As in the case of female eunuchoids, male eunuchoids with low FSH are of variable normal height, often with disproportionately long extremities and an increased span due to delayed epiphyseal closure. Although the male generally presents with the chief complaint of failure of full sexual development, he is often troubled by lack of physical vigor and increased fatiguability. The latter complaints are minimal or absent in the women with this condition. In 2 of the women, libido was said to have been normal; in males it is uniformly deficient. Physical findings are more striking in the males, there being little or no recession of the hairline at the temples, almost no beard, little body hair, and decreased growth of the thyroid cartilage with consequent lack of vocal depth. Axillary and pubic hair are diminished and comparable in amount to that of the female patients. The genitalia show either no development or slight pubertal changes with some penile and scrotal growth. The testes are similar to those of prepubertal or early adolescent males, both by palpation and by biopsy. Muscular development is usually poor. An occasional patient may be excessively obese, and there may be a tendency toward localization of the fat in the hips and breasts. Some of these males have a peculiarly waxy skin, and in some there is fine wrinkling about the eyes and mouth; this was seen in only 1 such female patient, and then to a lesser degree.

As in the females, the only laboratory findings of significance are a low or negative titer of urinary FSH and a 17-ketosteroid excretion often lower than would be expected in a castrated male of the same age. On radiologic examination, evidence of mild osteoporosis is not uncommon; bone age is

moderately retarded in the younger patients. In some cases there is vertebral epiphysitis; the vertebrae are more shallow than normal with notching of the anterior portions of their superior and inferior borders. Epiphysitis may result from any condition causing delayed closure of the vertebral epiphyses.

EFFECT OF ADMINISTRATION OF SEX HORMONES IN CASES OF EUNUCHOIDISM WITH LOW FSH

Male and female eunuchoids with low FSH respond to the administration of androgen or estrogen, respectively, by development of the appropriate secondary sexual characteristics. This response indicates that the failure of development is not due to end-organ refractoriness to sex hormones.

Administration of estrogen to the 4 female eunuchoids with low FSH in each instance led to development of the breasts, an increase in axillary and pubic hair, maturation of the genitalia, and more feminine contours. In each, cyclic administration of estrogen resulted in withdrawal bleeding. When estrogen was given continuously, courses of progesterone given every five weeks led to vaginal bleeding, and the uterus was accordingly assumed to be normal. Prolonged discontinuance of estrogen in each of these 4 patients was followed by cessation of vaginal bleeding, and the titers of FSH remained below those found in normal adults.

In male eunuchoidism with low FSH, administration of testosterone leads gradually to the growth of normal amounts of body and sexual hair, balding at the temples, growth of the thyroid cartilage with deepening of voice, and growth of the phallus. The testes remain small. There is improved muscular development and frequently disappearance of excessive fat. The sense of well-being increases, as does libido. Testosterone therapy does not lead to spermatogenesis in these patients,[1] although such results have been reported in cases said to have been eunuchoidism with low FSH (12, 13) but in which the diagnosis may have been delayed puberty. It is possible that pituitary secretion began or increased coincidentally, or was stimulated by the administration of testosterone, and acted along with testosterone to produce spermatogenesis. Treatment should be discontinued periodically in such patients and urinary FSH re-assayed after a suitable interval in order to ascertain whether or not the pituitary has begun to function normally.

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[1] Administration of testosterone to hypophysectomized male rats may permit maintenance, or lead to development of the seminiferous tubules and to the production of sperm (14, 15, 16). Similar results have not been observed in the hypophysectomized monkey (16) or in human subjects with panhypopituitarism.

EFFECT OF ADMINISTRATION OF HUMAN CHORIONIC GONADOTROPIN (HCG) IN CASES OF EUNUCHOIDISM WITH LOW FSH

In hypophysectomized male rats, administration of human chorionic gonadotropin in large doses can induce and maintain normal testicular function (16, 17, 18); functioning Leydig cells appear which lead to production of androgen and growth of secondary sexual characteristics; the seminiferous tubules mature and produce sperm.

In human male eunuchoids with low FSH, the administration of HCG in large doses over prolonged periods of time leads, similarly, to qualitatively normal testicular function, masculinization, and in some cases fertility (19).

In the hypophysectomized female rat, doses of HCG which effect testicular development in the male are almost entirely ineffective (20, 21); there is no follicular enlargement and only slight theca-luteinization. With very large doses, however, some stimulation may be obtained (20).

Despite the demonstrated refractoriness of hypophysectomized female laboratory animals to HCG, it was decided to test the effect of HCG in the human female eunuchoid with low FSH in an attempt to duplicate the results of successful experiments in animals and the successful outcome of prolonged injection in the human male.

In our patients who were treated with HCG, full secondary sexual development had occurred during prior treatment with estrogen, so that the appearance of menstruation was the major criterion for the efficacy of HCG therapy.

Patient C. W. (see Appendix) received 5,000 units of HCG subcutaneously twice weekly for eleven and a half months. At the end of five months of therapy no bleeding had occurred. A five-day course of progesterone given at this time failed to induce menstruation. HCG was continued for another five months, during which time amenorrhea continued. FSH (Armour) in a dosage of 50 Armour units daily, was given along with HCG for one month. No bleeding occurred when the FSH was discontinued, nor following withdrawal of HCG after another fifteen days.

Patient H. L. was given HCG in a dosage of 5,000 units twice weekly for eight months. No vaginal bleeding occurred during this time, or at the end of the course when progesterone was given along with HCG for five days.

In the hope of inducing ovulation and conception in the only married patient in this group (M. F.), stilbestrol and HCG were given concurrently for twelve months. The HCG was given three times weekly in a dosage of 10,000 units per injection, and the stilbestrol in a daily oral dosage of 1 milligram for five-week periods alternating with ten-day periods of om's-

sion. During each period without stilbestrol menstrual bleeding occurred, but there was no conception, nor was there evidence that HCG had an effect any different from that of stilbestrol alone. HCG was then continued for an additional three and a half months without estrogen. During the last two months of this period ACTH-gel in a dosage of 20 units was given in addition to HCG, but there was no conception, menstruation, or other effect, except an increase in 17-ketosteroid excretion.

EFFECT OF ADMINISTRATION OF FSH AND PREGNANT MARE'S SERUM IN CASES OF EUNUCHOIDISM WITH LOW FSH IN THE FEMALE

As noted previously, 1 of our patients received FSH together with HCG for thirty days, but no menstruation followed this course of therapy. The same patient had previously received pregnant mare's serum (primarily FSH-like gonadotropin) in a dosage of 300 units intramuscularly for thirty days. During the last five days of this period progesterone was also administered. No bleeding followed withdrawal of these substances.

An attempt was made to administer FSH subcutaneously to Patient M. F., but a severe allergic reaction occurred at the sites of injection, and therefore FSH was discontinued. This patient proved to be sensitive to an intradermal test-dose of pregnant mare's serum, which therefore was not administered.

17-KETOSTEROID EXCRETION IN EUNUCHOIDISM WITH LOW FSH

In 2 of the female eunuchoids with low FSH, as well as in most of the 9 female patients with high FSH (as in most cases of gonadal dysgenesis), 17-ketosteroid excretion was lower than expected for the age and sex (Table 2). Pre-treatment 17-ketosteroid levels in male eunuchoids with low FSH are also lower than might be expected, even allowing for the absence of gonadal contribution to 17-ketosteroid excretion.

Adrenocorticotropin (ACTH) is known to stimulate the production of adrenal androgen (nitrogen-retaining or N-hormone). To account for the increase in the excretion of 17-ketosteroids of adrenal origin which occurs at puberty the theory has been advanced that LH may be synergistic with ACTH in producing adrenal androgen (4, 8). An alternative hypothesis is that the pituitary secretes two types of ACTH—one stimulating corticoid production primarily, and the other appearing at puberty and stimulating the adrenal to produce androgenic substances excreted as 17-ketosteroids (22). It was demonstrated that ACTH deficiency could not account for the low 17-ketosteroid excretion in the 4 female eunuchoids without FSH; in none was there deficient ACTH production as judged by sugar tolerance curves, eosinophil counts, or measurement of urinary 11-oxysteroids in several patients.

TABLE 2. EFFECTS OF TREATMENT WITH HUMAN CHORIONIC GONADOTROPIN AND WITH ESTROGEN ON 17-KETOSTEROID EXCRETION IN FEMALE PATIENTS WITH EUNUCHOIDISM AND NORMAL STATURE

Patient	No treatment		HCG therapy		Estrogen therapy	
	No. of determinations	17-KS (mg./24 hrs., avge.)	No. of determinations	17-KS (mg./24 hrs., avge.)	No. of determinations	17-KS (mg./24 hrs., avge.)
Low FSH						
C.W.	20	6.1	18	4.7	7	8.1
M. DiM.	1	3.3				
H.L.	1	6.8	4	5.3	1	1.8
M.F.	3	3.7	2	3.4	3	2.4
High FSH						
M.G.	3	5.1				
E.C.	6	2.4				
A.B.	2	7.8				
E.S.	1	5.4				
R.H.	2	8.4				
I.S.	2	1.4	6	0.9	12	1.2
G.M.	2	3.6				

The effect of estrogen on adrenal activity has been extensively studied in the rat, but results are equivocal; some investigators report increases and others report decreases in adrenal weight and activity (reviews, 23, 24). The administration of estrogen led to a significant increase in urinary 17-ketosteroids in only 1 of the 3 female eunuchoids with low FSH in whom 17-KS excretion was studied. The administration of estrogen was, however, associated with an increased growth of pubic and axillary hair in 3 patients.

There was no rise in 17-ketosteroid excretion during treatment with HCG. Although HCG is thought to be similar to LH, this failure cannot be used against the theory that LH itself enhances production of adrenal N-hormone. The low levels of urinary 17-ketosteroids in these patients therefore remain unexplained.

DISCUSSION

The clinical and laboratory similarities are striking in a comparison of the syndrome of eunuchoidism with low FSH in the female and what would appear to be the counterpart of the condition in the male. In both, the gonad is entirely inactive, the values for urinary 17-ketosteroids are subnormal relative to the values obtained in castrates, and there is no measurable urinary FSH. In both, the basic fault appears to be in the pituitary.

One striking difference is in the strong response of the male to HCG therapy, and the refractoriness of the female. This difference in responsiveness to HCG is also seen in hypophysectomized male and female rats.

In both males and females with this syndrome it is not known whether the inability to produce gonadotropins is specific for FSH or whether it extends to other gonadotropins. Since LH is capable of inducing some testicular function in male laboratory animals, the absence of testicular development in untreated human male eunuchoids with low FSH suggests that these patients are in fact eunuchoids without either FSH or LH. As studies in mice, rats and rabbits indicate that hormone production by the ovary is induced only by a combination of FSH, LH and LTH, the absence of evidence of ovarian function in the untreated female does not tell us whether the deficiency concerns FSH alone or whether it involves all the gonadotropins. That Heller (25) has succeeded in inducing some degree of maturation and vaginal cornification in such patients by injection of purified FSH suggests that their pituitaries are capable of producing LH.

On the other hand, in Patient M. DiM. no measurable LH could be detected in an assay using the weight of the ventral prostate of the hypophysectomized male rat. It is possible that the pituitary is capable of producing LH if stimulated by some other administered hormone.

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APPENDIX

FOUR CASES OF FEMALE EUNUCHOIDISM WITH LOW URINARY FSH

Case 1

C.W. (MGH #229892) (Fig. 1) was first seen in 1939 at the age of 18 because she had not yet menstruated. She had always been in excellent health and was an adequate student. Although no records were available, growth was said to have been at a normal

rate. Pubic hair had appeared two years previously; there had been no growth of axillary hair and no development of the breasts.

The patient's mother had first menstruated at the age of 17; her height was 67 inches, as was that of the father. A married sister, aged 28, was 68 inches tall and had had 2 children. A 30-year-old brother was 72 inches tall and was said to have matured normally. All members of the family were slender.

On examination the patient appeared well and active. Her height was $68\frac{1}{2}$ inches, span $68\frac{3}{4}$ inches, and weight 103 pounds. There was complete absence of breast tissue and no development of the areolae or nipples. The clitoris was not enlarged; no uterus could be felt by rectal examination. The remainder of the physical examination showed nothing abnormal, except for moderate myopia with myopic crescents of the optic fundi.

Routine examinations of the blood and urine yielded normal results. Radiologic examination of the skull showed no abnormality of bone formation or density, and the sella turcica was of normal size. Bone age, estimated from radiographs of the hand corresponded to the chronologic age. The glucose tolerance curve was normal.

In January 1943 an exploratory laparotomy was performed by Dr. Joseph Meigs. A normal uterus measuring only 5 cm. and normal tubes were seen. Each ovary measured approximately 2 cm. in length and 0.5 cm. in thickness. In one, from which a biopsy specimen was taken, there appeared to be a tiny follicle. A specimen also was taken from the other ovary, in which no follicles were seen grossly. On histologic examination several small follicles were seen in each specimen (Fig. 2).

In 1947, thyroid studies and an insulin tolerance test were performed; the results were normal. Repeated assays for urinary FSH were negative at 3 and 6.5 m.u. levels; the average urinary 17-ketosteroid excretion was 6.1 mg. per twenty-four hours.

Between October 1941 and August 1942, the patient was treated with estrogen. During this time there was gradual development of the breasts, growth of axillary and pubic hair, and closure of the wrist epiphyses. Menstruation occurred after two and a half months of estrogen therapy and recurred during subsequent periods when estrogen was withdrawn.

Between 1943 and 1950, estrogen in varying dosages was given, usually in courses of five weeks alternating with ten days of no medication. Withdrawal bleeding occurred regularly. Therapy was discontinued on several occasions without the appearance of spontaneous menstruation. In 1950 the studies described in the text were begun.

The patient has been well except for ophthalmologic difficulties with myopia and retinal separations, which first occurred in 1946. Her present height is $69\frac{5}{8}$ inches, span $71\frac{3}{4}$ inches, and weight 115 pounds. She is again taking estrogen, but recently withdrawal bleeding has been infrequent.

Case 2

H.L. (MGH #719481) (Fig. 1), a 26-year-old graduate nurse, was admitted for study of amenorrhea in 1950. Her general health was excellent, but menstruation had failed to occur and her breasts had never developed. She had been tall and slender at age 16, but had gained weight to 190 pounds in the next two years. At age 21 she was treated with an anterior pituitary extract for a three-month period without effect on the breasts and without the occurrence of menstruation, but during this time her weight decreased to 130 pounds. Subsequently her weight varied between 125 and 140 pounds. In 1946 she received stilbestrol for approximately three weeks, but stopped this medication because of nausea; there was no menstrual bleeding. Three weeks prior to admission the patient strained her back lifting a heavy object, and radiologic examinations were said to have shown unfused epiphyses and osteoporosis. The patient believed she was still growing at

the time of admission. The history was otherwise unremarkable, except for a painful osteoma of the left tibia which had been removed in 1946.

Two siblings, aged 24 and 30, were apparently normally developed.

The patient appeared to be her stated age, and in good health. She was 67½ inches tall, with a span of 69½ inches, and weighed 128 pounds. Blood pressure was 110/75 mm. Hg. The breasts were slightly enlarged, but no glandular tissue could be felt. Axillary and pubic hair was present but sparse. The genitalia were underdeveloped, and the uterus could not be felt by rectal examination. On ophthalmologic examination there was no ocular evidence of pituitary tumor.

The urine was normal. The hemoglobin level, and erythrocyte and leukocyte counts were normal; there were 50 and 95 eosinophils per cu. mm. on two direct counts. The serum non-protein nitrogen level was 15 mg. per 100 cc, and the serum phosphorus level 5.3 mg. per 100 cc. An insulin tolerance test showed a fasting blood sugar level of 89 mg. per 100 cc. with a fall to 68 mg. in thirty minutes, a rise to 76 mg. in sixty minutes, and a return to 89 mg. in ninety minutes.

The teeth were unremarkable by radiologic examination. Examination of the thoracic and lumbar spine revealed moderate osteoporosis without collapse of any vertebral body and without expansion of the intervertebral discs. There was no "epiphysitis," although there was minimal wedging of several midthoracic vertebrae. An intravenous pyelogram showed that the kidneys were normal in size, shape and position, and that the excretion of Diodrast was normal.

The patient was given stilbestrol in a dosage of 2 mg. daily, to be taken for four-week periods with a one-week interval between courses. During withdrawal periods she noted only vaginal spotting after the first three courses; after ensuing courses, normal withdrawal bleeding occurred. Under this program of treatment there was considerable enlargement of the breasts and an increase in the growth of axillary and pubic hair. During stilbestrol therapy, another insulin tolerance test yielded normal results, and the serum phosphorus level was 4.7 mg. per 100 cc.

After this regimen had been continued for eight months, stilbestrol was stopped for three months, during which time three determinations of urinary FSH were negative for 6.5 mouse units per twenty-four hours. The studies described in the text were then performed. Subsequently, the patient resumed cyclic estrogen therapy with regular withdrawal periods and has continued good health.

Case 3

M.DiM. (MGH #459716) (Fig. 1), a 36-year-old hairdresser, was admitted in 1949 because of an alleged red blood count of 3.9 million per cu. mm. and suspected thioglycollic-acid toxicity. The patient's only complaint was of increased fatigue during the preceding five years, during which time she had been giving "cold-waves" with thioglycollate. She had never menstruated, her breasts had not developed, and axillary and pubic hair growth had always been scant. Sexual interest was said to be normal.

Seven siblings, aged 23 to 39, were said to be well and normally developed.

On physical examination the patient was a pleasant, somewhat shy woman with long extremities. Her height was 64 inches, span 71 inches, and weight 112 pounds. Blood pressure was 120/80 mm. Hg. The breasts and the external genitalia were completely undeveloped. The uterus was palpable but small, as were the vagina and cervix. Axillary and pubic hair was scanty. The back was moderately kyphotic. There was moderate exophthalmos, said to be congenital and familial.

Repeated hemoglobin determinations yielded readings within normal limits. The white blood cell count, differential count and platelet count were normal; bone marrow smears showed nothing unusual. Results of urinalysis and of liver function studies were

normal. Assay of urinary FSH was negative for 6.5 mouse units, and the urinary 17-ketosteroids measured 3.3 mg. per twenty-four hours. A radiograph of the skull showed unusual thickness of the dorsum sellae and extensive pneumatization of the maxillary and ethmoid cells. The sella turcica was normal in size; the pineal was calcified and normal in position. A lateral film of the spine showed moderate kyphosis with some flattening of the upper dorsal vertebrae, but no "epiphysitis." All the visualized vertebrae appeared to be moderately demineralized, except for the eighth thoracic vertebra, in which there were changes consistent with Paget's disease.

The patient was given stilbestrol in a dosage of 1 mg. daily to be taken for 35-day periods with a one-week interval between courses. Vaginal bleeding occurred whenever stilbestrol was stopped. During one and a half years of this therapy she noted only slight breast development and no subjective changes. There was no change in axillary or pubic hair. Libido was allegedly unchanged. Because of the slight effect and because the patient considered menstruation to be a nuisance, she discontinued medication and hospital visits. She did not return until 1955. When last seen she was asymptomatic. Her height was unchanged, as were the radiographic findings in her spine. Almost no breast tissue was palpable.

Case 4

M.F. (MGH #739966) (Fig. 1) was first seen in 1951 at the age of 28 because of pain in the nipple of the left breast. No cause for pain was found in the breasts, which were completely undeveloped, and the symptom soon disappeared. The patient had never menstruated, although she had allegedly experienced vaginal bleeding on one occasion shortly after her marriage one year earlier. At the age of 15, because of failure to develop or to menstruate, the patient received injections and pills of unknown type, which were without effect. No subsequent treatment had been given. An appendectomy had been performed at the age of 24, at which time the ovaries were described as being present but infantile in appearance. No biopsy was performed. Axillary and pubic hair had developed at the normal time. Intercourse was described as having been painful because of a small introitus, but satisfaction was allegedly complete.

On physical examination the patient was a cheerful, somewhat obese young woman weighing 152 pounds. Her height was 62½ inches, span 65¼ inches, and blood pressure 120/88 mm. Hg. Subareolar fat was present, but no breast tissue was palpable, and the nipples and areolae were undeveloped. There was a small amount of axillary hair and a moderate amount of pubic hair. The vagina was short, the cervix small, and the uterus could not be felt. Results of the physical examination were otherwise negative.

A radiograph showed the skull to be normal; the sella turcica was normal in size and there was no erosion of the clinoid processes. Upon lateral examination of the lumbar spine, the vertebrae were normal in density and contour. Films of the hands showed that all of the epiphyses were closed.

With an 800-calorie diet and the help of methamphetamine, the patient's weight was reduced to 122 pounds. During estrogen therapy she experienced withdrawal bleeding, her breasts developed and there was an increase in axillary and pubic hair. She stated that she felt somewhat better while taking estrogen.

Following stilbestrol and HCG therapy (see text) the patient received no treatment for seven months, whereupon she noted the return of hot flashes, which she had also experienced briefly in 1941. During the period of hot flashes and no medication, the titer of urinary FSH was repeatedly negative for 6.5 mouse units per twenty-four hours. The hot flashes responded to estrogen therapy, which has been continued up to the present time.

URINARY ESTROGEN EXCRETION IN CUSHING'S SYNDROME*

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ABSTRACT

Since overproduction of estrogens could conceivably play a role in the pathologic physiology of Cushing's syndrome, an attempt was made to isolate, identify, and quantitate the urinary estrogens excreted by a 58-year-old postmenopausal woman with metastatic adrenocortical carcinoma and Cushing's syndrome.

By means of countercurrent distribution and paper chromatography, estrone and estriol were identified and isolated in a fluorometrically homogeneous form. During the control period the patient excreted 5.7 micrograms of estrone and 34.2 micrograms of estriol daily as measured by photofluorometry. Upon the administration of ACTH, the excretion of estrone increased 12-fold and that of estriol 5-fold. The isolated estrogens were assayed for biologic activity and the positive results were in reasonable agreement quantitatively with the fluorometric measurements. The acetate derivatives of the isolated estrogens were prepared, and their behavior upon paper chromatography was found to be identical with that of appropriate reference standards of authentic estrogen acetates.

No estradiol-17β was found in the control urine, but traces were detectable by paper chromatography during the ACTH period.

The relatively high urinary excretion of estrone and estriol by this postmenopausal woman with adrenocortical carcinoma and the increased estrogen excretion with ACTH stimulation suggest that these estrogens or their precursors were produced by the adrenocortical carcinoma and that this function of the tumor tissue was not autonomous.

ACCORDING to reports in the literature, some patients with adrenocortical hyperfunction excrete excessive amounts of estrogens in the urine, whereas others excrete normal or decreased amounts. The pathophysiologic significance of these observations is unknown. As early as 1933, Frank reported high levels of estrogenic material in the urine of patients with Cushing's syndrome due to adrenocortical carcinoma, but low or normal levels in those with benign cortical hyperplasia or adenoma

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(1). On the basis of a limited number of observations, he suggested that the level of urinary estrogens might be used to distinguish between malignant and benign cases. Unfortunately, insofar as a reliable differential test is concerned, it was later demonstrated that not all patients with adrenocortical carcinoma excrete excessive amounts of estrogens (2–8). Furthermore, patients with benign adrenocortical hyperplasia and adenoma may also excrete large amounts of estrogens (9, 10).

In nearly all of these investigations the urinary estrogens were determined by biologic methods and, with 1 exception, were not isolated and identified chemically. In 1945, Mason and Kepler were successful in isolating 62 mg. of crystalline estrone from a 26-day collection of urine from a woman with Cushing's syndrome and adrenocortical carcinoma (11). They searched for estradiol and estriol but failed to find any.

Since obvious physical signs of hyperestrogenism rarely occur in patients with Cushing's syndrome, it would appear unlikely that estrogens play a major role in the pathologic physiology of Cushing's syndrome. However, disturbances in estrogen metabolism could conceivably be involved in the production of some of the manifestations of Cushing's syndrome, such as atrophy of the gonads and accessory sex organs with suppressed sexual function in the male, retention of sodium chloride and water, and disturbances in fat metabolism. Although these findings may well be due exclusively to other hormonal abnormalities, the possibility that estrogens may also play an important role stimulated the present investigation.

In this study an attempt was made to identify the urinary estrogens in a patient with adrenocortical carcinoma and Cushing's syndrome, and to ascertain whether the urinary estrogens in Cushing's syndrome represent metabolites of corticoids or true secretory products of the adrenal cortex. The effect of administration of adrenocorticotropin (ACTH) upon estrogen excretion in this patient was also studied.

CLINICAL MATERIAL AND METHODS

Case report

The patient was a 58-year-old postmenopausal woman who first experienced progressively severe weakness in March 1954. Hypertension, weight loss, truncal obesity, moon facies, and pitting ankle edema developed during the next year. In April 1955, a large tumor of the left adrenal gland was removed surgically. The microscopic pathologic diagnosis was adrenocortical carcinoma.

The patient recovered from the operation uneventfully and improved postoperatively until December 1955, when all of her previous symptoms and signs gradually recurred. In addition, hepatomegaly developed and x-ray examination revealed pulmonary metastases. The positive diagnostic laboratory findings included a diabetic type of glucose tolerance curve and elevated urinary 17-ketosteroids (32.6 mg. per twenty-four hours) and 17-hydroxycorticosteroids (25.0 mg. per twenty-four hours).

The estrogen study was carried out at this time. A control urine was collected for five days. An aliquot equivalent to four fifths of a single day's collection was analyzed for estrogens, as described later. The patient was then given 240 units of Armour's ACTH-gel intramuscularly daily for four days, during which period all the urine was collected. An aliquot equivalent to four fifths of a single day's collection was analyzed for estrogens. The remainder of each urinary pool was used for other analyses.

Subsequently, the patient was treated with amphenone and 6-mercapto-purine, but she failed to improve and died with septicemia.

Postmortem examination revealed carcinoma of the left adrenal cortex with metastases to the lungs, liver, and retroperitoneal tissues. There was a lobular pneumonia of the right lung. The ovaries and the one remaining adrenal gland were small and atrophic. Microscopically, there was no histologic evidence of hormonal function. The uterus was normal in size and contained an endometrial polyp measuring 3.5 cm. in diameter. The endometrium appeared to be in the proliferative phase.

Methods

The urinary glucuronisides were hydrolyzed with β-glucuronidase and extracted continuously with ether, as described by Gallagher *et al.* (12). The phenolic fractions were obtained by partitioning between toluene and 1*N* NaOH by a modification of Engel's method (13). The phenolic fractions of all urine samples were subsequently analyzed, as indicated in the flow sheet (Fig. 1). Mitchell's method of separating estrone and estradiol-17β from estriol by partitioning between water and benzene was used (14).

Initial countercurrent distributions of 99 transfers were carried out on the benzene-soluble fractions to separate estrone from estradiol-17β, and on the aqueous fractions to separate estriol from other phenolic fluorogens. The solvent systems and the number of transfers (n) are indicated in the flow sheet (Fig. 1). All countercurrent distributions

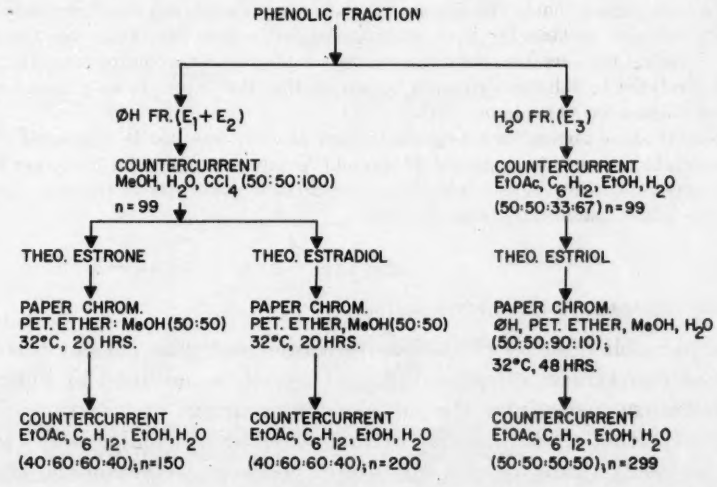

FIG. 1. Scheme for the routine fractionation of urinary phenolic fractions. E_1 = estrone, E_2 = estradiol-17β; E_3 = estriol-17α,16β; n = number of transfers. All solvent ratios are by volume.

were carried out in an all-glass, 100-tube instrument using the methods developed by Craig (15) as adapted for estrogens by Engel (13).

Following distribution, the content of each tube was analyzed for estrogens by photofluorometry using the methods of Bates and Cohen (16) as modified by Engel (13). A Farrand photofluorometer (model A) was used, with Corning glass filters #3389 and #5113 (436 mμ max.) as the lamp filter and Corning glass filters #3387 and #4308 plus an interference filter ((485 mμ) as the photocell filter. Theoretical distribution curves for each of the estrogens were calculated, as described by Craig and Craig (17). The degree of agreement between the analyzed and theoretical distribution curves was used as a criterion for the demonstration of homogeneity and identity of the isolated estrogens.

Following the initial countercurrent distributions, the contents of the tubes (which theoretically contained each one of the individual estrogens) were combined and analyzed by paper chromatography using the techniques developed by Bush (18). The solvent systems, temperature, and time of development are indicated in the flow sheet. Appropriate reference standards of authentic estrogens were run simultaneously, and material corresponding to the reference standards was eluted from the test paper chromatograms. The position of the estrogens on the paper chromatograms was detected with an aqueous solution of 1 per cent ferric chloride and 1 per cent potassium ferricyanide which reacts with phenols to give a permanent blue-green stain (19).

A second countercurrent distribution was performed on each of the estrogen fractions obtained by paper chromatography using the solvents and number of transfers indicated. Usually the individual estrogens were obtained in a fluorogenically homogeneous form after the second countercurrent distribution.

To obtain additional evidence for identification, the contents of the tubes making up each estrogen peak from the second countercurrent distribution were combined; one aliquot of each pool was acetylated, and another was tested for biologic estrogenic activity. Acetylation was carried out with acetic anhydride and pyridine at room temperature for eighteen hours. The acetate derivatives were analyzed simultaneously with authentic estrogen acetates by paper chromatography in petroleum ether and methanol at 24° C. Before the acetates could be detected on the paper chromatograms, they had to be hydrolyzed to the free estrogens by suspending the papers in an atmosphere of HCl and dioxane for three hours. (20).

Two methods of testing for estrogenic biologic activity were used—one based on the uterine-weight response of castrated 21-day-old female rats (21), and the other based on the response of the vaginal epithelium of castrated adult rats to the test material which was administered intravaginally (22).

RESULTS

Urinary estrogens in the control period

The phenolic fraction of the control urine from this patient was first analyzed for estrone, estradiol-17β, and estriol, as outlined in Figure 1. The resulting curve from the initial countercurrent distribution of the benzene fraction of the phenolic extract revealed two major peaks which were obviously made up of a mixture of fluorescent compounds, since a theoretical curve for a pure compound could not be drawn which would fit the observed curve (Fig. 2A). The possibility that estrone was one of the fluorescent compounds in the less polar peak, and estradiol-17β one of those in the more polar peak was examined further.

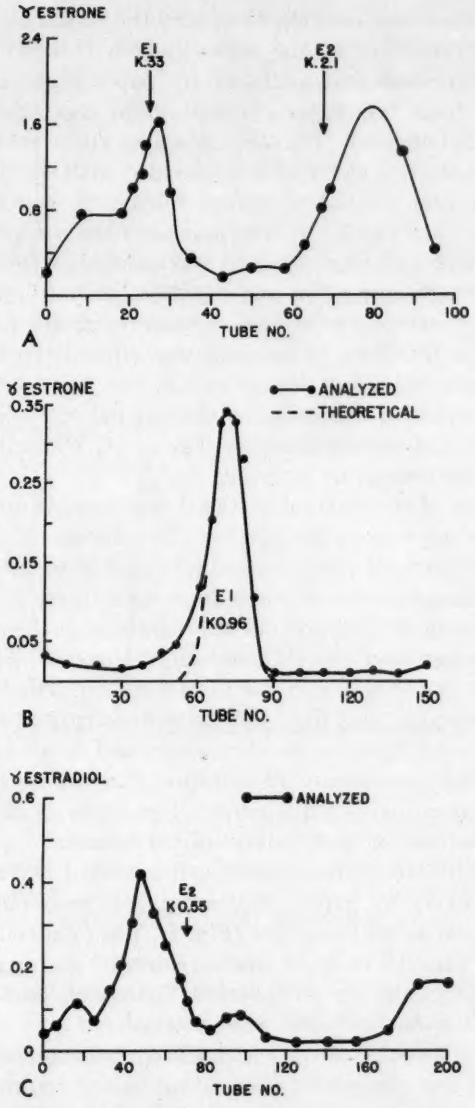

FIG. 2. Countercurrent distributions of control urine for estrone (E_1) and estradiol (E_2). ●——● analyzed curves; – – – theoretical curve for estrone. **A.** Initial countercurrent distribution of the benzene fraction of the phenolic extract. MeOH, H_2O, CCl_4 (50:50:100); n = 99; partition coefficient (K) for $E_1 = 0.33$; $K_{E2} = 2.1$. **B.** Countercurrent distribution for estrone following paper chromatography. EtOAc, C_6H_{12}, EtOH, H_2O (40:60:60:40); n = 150. $K_{E1} = 0.96$. **C.** Countercurrent distribution for estradiol-17β following paper chromatography. EtOAc, C_6H_{12}, EtOH, H_2O (40:60:60:40); n = 200. $K_{E2} = 0.55$.

Identification of estrone (control). Following the foregoing countercurrent distribution, the contents of the tubes (which theoretically contained estrone) were combined and analyzed by paper chromatography. The estrone fraction from the paper chromatogram was again analyzed by countercurrent distribution (Fig. 2B). A single fluorescent peak was observed in the distribution curve which coincided with the theoretical curve for estrone. The total amount of estrone calculated from the countercurrent fluorometric data was 5.7 micrograms per twenty-four hours.

Since the amount of estrone obtained was insufficient for both the preparation of the acetate derivative and bioassay, only a bioassay was performed. When a total dosage of 0.06 microgram of the isolated material (in fluorometric equivalents of estrone) was administered intravaginally to each of 14 castrated, adult female rats in two fractional doses on successive days, complete cornification of the vaginal epithelium occurred in all 14 rats but in none of the controls. The positive reactions indicated a qualitative test for estrogenic activity.

Another aliquot of the isolated material was assayed quantitatively for estrone by the rat uterine-weight method. Two dosages (0.2 and 0.5 microgram) of the test material were injected into each of 6 castrated immature rats in six fractional doses on three successive days. A standard dose-response curve using 0.1, 0.2 and 0.5 microgram of authentic estrone with 5 rats at each dosage level was obtained simultaneously. The animals were sacrificed on the fourth day and the uteri weighed. The biologic activity observed in the estrone peak from the last countercurrent distribution was equivalent to 4.8 micrograms of estrone excreted in a 24-hour period, as compared with 5.7 micrograms determined fluorometrically.

Identification of estradiol-17β (control). The contents of the tubes from the initial countercurrent distribution of the benzene fraction (Fig. 2A), which theoretically would have contained estradiol, were combined and analyzed successively by paper chromatography and countercurrent distribution, as shown in the flow sheet (Fig. 1). The final countercurrent distribution curve (Fig. 2C) revealed that no estradiol was excreted during the control period, although an unidentified fluorescent material which was slightly more polar than estradiol was observed.

Identification of estriol (control). A single nonhomogeneous peak of fluorescent material was observed following the initial countercurrent distribution of the aqueous fraction of the phenolic extract (Fig. 3A). To separate estriol from this mixture of fluorescent compounds, the contents of the tubes which theoretically contained estriol were combined and analyzed by paper chromatography. The estriol fraction from the paper chromatogram was again examined by countercurrent distribution. A single homogeneous peak of fluorescent material which coincided with the theo-

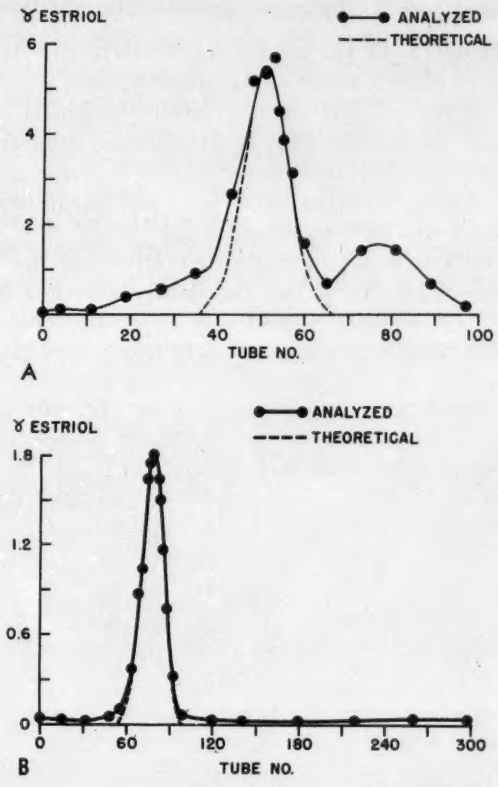

FIG. 3. Countercurrent distribution of control urine for estriol (E_3). ●——● analyzed curves; – – – theoretical curves for estriol. **A.** Initial countercurrent distribution of water-soluble fraction of the phenolic extract. EtOAc, C_6H_{12}, EtOH, H_2O (50:50:33:67): n = 99: $K_{E3} = 1.2$. **B.** Countercurrent distribution for estriol following paper chromatography. EtOAc, C_6H_{12}, EtOH, H_2O (50:50:50:50); n = 299: $K_{E3} = 0.35$.

retical curve for authentic estriol was observed in the distribution curve (Fig. 3B), identifying the material as estriol. From these data, the amount of estriol excreted daily by the patient was calculated to be 34.2 micrograms.

An 8-microgram portion of this material was acetylated and analyzed by paper chromatography. The acetate derivative had the same mobility as authentic estriol triacetate.

Another aliquot was assayed for biologic activity by the uterine-weight method. The bioassay showed that the material had estrogenic activity, quantitatively equivalent to a daily estriol excretion of 34.2 micrograms.

Urinary estrogens during ACTH therapy

The urine collected while the patient was treated with ACTH was analyzed in exactly the same manner as the control urine.

Analysis for estrone (ACTH period). After the initial countercurrent distribution, paper chromatography, and the second countercurrent distribution, estrone was obtained from the urine in a fluorogenically pure form. From the countercurrent distribution data, it was calculated that the daily excretion of estrone was 69.3 micrograms.

The isolated estrone and its acetate derivative had the same mobility upon paper chromatography as had the reference standards of authentic estrone and estrone acetate, respectively (Fig. 4). The same material caused full cornification of the vaginal epithelium in 14 out of 14 castrated

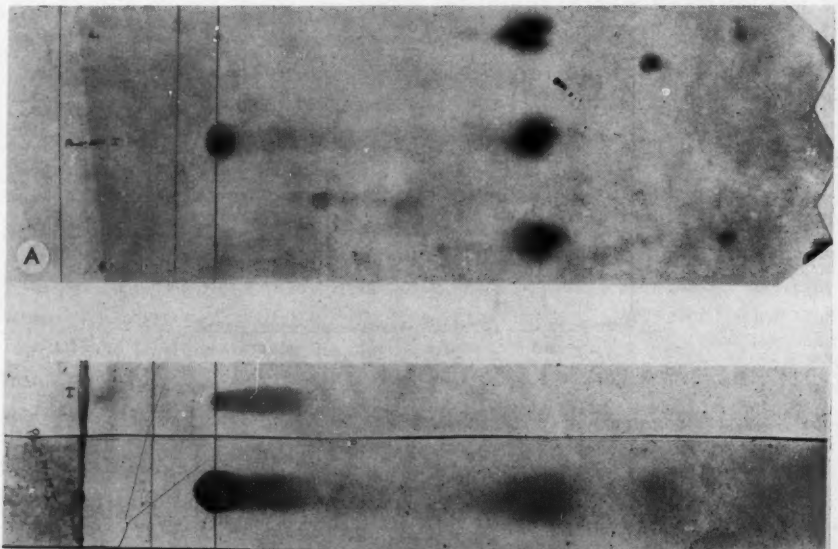

FIG. 4. Paper chromatography of the estrone fraction from the second countercurrent distribution (on ACTH). **A.** Photograph of paper chromatogram of the isolated estrone (middle spot) with estrone reference standards on each side, developed in petroleum ether-methanol (1:1) for eighteen hours of 32° C. **B.** Photograph of the paper chromatogram of the acetate derivative of the isolated estrone (middle) with reference standards of authentic estrone acetate on the left and of free estrone on the right, developed in petroleum ether-methanol (1:1) for one and a half hours of 24° C. (The large dark spot at the origin is due to impurities in the extract which take the nonspecific ferric chloride-ferricyanide stain.)

adult female rats when administered intravaginally in a dose of 0.08 microgram per rat. Quantitative bioassay by the rat uterine-weight method previously described indicated a daily estrone excretion of 36.0 micrograms.

Analysis for estradiol-17β (ACTH period). Following the initial countercurrent distribution of the benzene fraction, paper chromatography, and a second countercurrent distribution, there did not appear to be any estradiol. However, small amounts could have been concealed by other fluorogenic substances in the final distribution. Therefore, the contents of the tubes which theoretically would have contained estradiol were combined and chromatographed on paper in a petroleum ether-methanol solvent system at 32° C. for nineteen and a half hours. A photograph of the developed

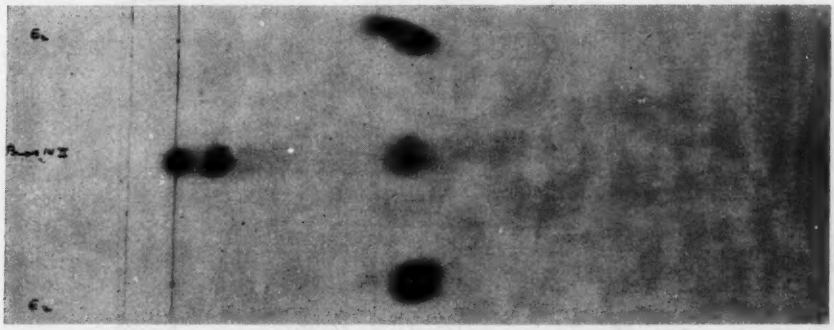

FIG. 5. Paper chromatographic analysis for estradiol-17β following second countercurrent distribution (on ACTH). Developed in petroleum ether-methanol (1:1) for nineteen and a half hours at 32° C. Middle spot is test material with 2 estradiol-17β reference standards on each side.

and stained paper chromatogram is shown in Figure 5. A spot with the same mobility as the 2 estradiol reference standards on each side became separated from that of other phenols on the paper chromatogram. Judging by the intensity of the staining of the spot, as compared with the reference standards, it was estimated that this spot represented about 2 micrograms of estradiol.

Analysis for estriol (ACTH period). Estriol was isolated from the urine collected during the ACTH period in a fluorogenically homogeneous form by the same methods used on the control urine. According to the calculations from the data of the final countercurrent distribution, the daily excretion of estriol amounted to 169 micrograms. The behavior of the isolated estriol and its acetate derivative upon paper chromatography was identical with that of authentic estriol and estriol triacetate, respectively. A bioassay of this material by the rat uterine-weight method indicated estrogenic activity equivalent to 63.4 micrograms of estriol excreted daily.

Summary of the effects of ACTH. The effects of administration of ACTH on the excretion of urinary estrogens by this patient with adrenocortical carcinoma are summarized in Figure 6. During the control period, the patient excreted 5.7 micrograms of estrone and 34.2 micrograms of estriol daily. With the administration of ACTH, the excretion of estrone increased to 69.3 micrograms daily, and that of estriol to 169 micrograms daily. Estradiol could not be demonstrated in the urine during the control period, but excretion during the ACTH period was approximately 2 micrograms daily, as estimated by paper chromatography.

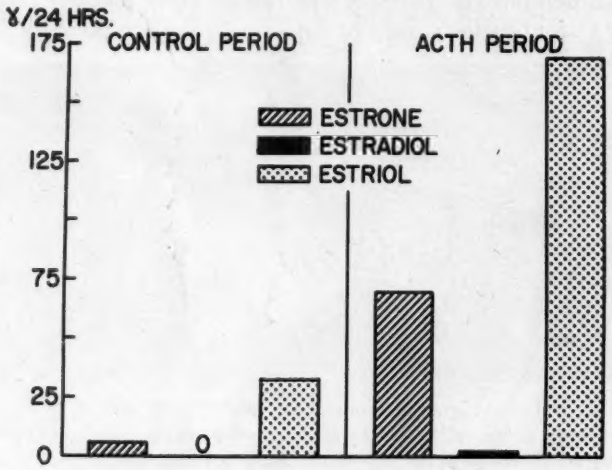

FIG. 6. Effect of administration of ACTH upon the excretion of urinary estrogens by a patient with adrenocortical carcinoma.

DISCUSSION

Before drawing any physiologic conclusions from these data, the criteria used in this investigation for the quantitative identification of urinary estrogens should be examined. It was demonstrated that the isolated compounds were phenols, by solvent partitioning between toluene and alkali and by the positive ferric chloride-ferricyanide color reaction. These phenols were fluorescent in concentrated sulfuric acid under the same conditions as authentic estrogens. In the identification of estrone and estriol, the final products had the same solubility characteristics as authentic estrogen standards upon multiple sequential analyses by paper chromatography and countercurrent distribution. The acetate derivatives of the isolated estrone and estriol had the same mobility as authentic estrone acetate and estriol triacetate, respectively, on paper chromatography. The final prod-

ucts were biologically active estrogens, and quantitatively the measurements by bioassay were in reasonable agreement with the values obtained for the same samples by fluorometry.

Thus, the quantitative identification of estrone and estriol rests on a firm foundation. This is not the case for estradiol. None could be demonstrated in the urine during the control period, and paper chromatographic evidence of only trace amounts was obtained during the ACTH period.

It would appear that the cancerous adrenocortical tissue in this patient produced considerable amounts of chemically identifiable estrogens, and that this estrogen production was increased by the action of the adrenocorticotropic hormone. This interpretation of the data is contrary to the classic concept that adrenocortical carcinomas are autonomous in their hormonal function.

Since the patient had intact ovaries and some non-neoplastic as well as neoplastic adrenocortical tissue, it is impossible to be certain that the cancerous tissue was the source of the urinary estrogens. However, it seems unlikely that the ovaries were the source of the urinary estrogens, since this patient was postmenopausal, and atrophic, nonfunctional ovaries were found at autopsy. Furthermore, the striking increase in urinary estrogens stimulated by the administration of ACTH strongly suggests that adrenocortical tissue was involved. To determine whether the neoplastic or the normal adrenocortical tissue was involved is the problem. At autopsy a single adrenal gland with atrophy of the cortex was found. It is doubtful that this cortical tissue could have responded to the amount of ACTH given to produce the observed increase in urinary estrogens. Even in patients with normal adrenal glands, increases in urinary estrogens of this order of magnitude upon the administration of ACTH have never been observed by the authors.

If the neoplastic adrenocortical tissue were the source of the urinary estrogens, the problem of whether these estrogens were true secretory products of the cancer or metabolites of some other precursor secreted by the cancer remains. Since ACTH produced a rise in urinary 17-hydroxycorticosteroids concomitant with the increase in estrogens in this patient, it is possible that the urinary estrogens were metabolites of the corticosteroids. To test this theory, a 600-mg. dose of hydrocortisone was administered intravenously to a woman with metastatic breast cancer who had undergone oophorectomy, adrenalectomy and hypophysectomy. The urine was collected during this time and analyzed for estrogens. No identifiable estrogens were demonstrated in the urine, suggesting that hydrocortisone, and probably other related corticosteroids, cannot be converted to estrogens to any significant degree, although the possibility of individual patient

variation must be taken into account. Whether or not the urinary estrogens were true secretions of the adrenal cancer or metabolites of some other precursors could not be determined by this investigation.

It should be emphasized that the results obtained in this patient and their physiologic implications are not necessarily applicable to all patients with adrenocortical carcinoma. Judging by past investigations, it would appear that the excretion of urinary estrogens is highly variable from patient to patient. The authors have recently investigated the excretion of urinary estrogens in a second patient with Cushing's syndrome due to adrenocortical carcinoma. No identifiable estrogens were isolated from the urine of this patient, even upon administration of ACTH.

That some patients have adrenocortical carcinomas which are not autonomous in their production of hormones has also been shown by Gallagher *et al.* (23), who demonstrated that the urinary excretion of neutral steroids can be altered in some patients by the administration of ACTH and corticosteroids. The stimulation of steroid production by exogenous ACTH and the suppression upon administration of corticoid suggest that endogenous ACTH may exert some control over the growth and function of the cancerous adrenocortical tissue in these patients. If this be the case, patients of this type might possibly benefit from hypophysectomy.

Acknowledgments

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THE METABOLISM OF EPINEPHRINE CONTAINING ISOTOPIC CARBON IN MAN*

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ABSTRACT

Schizophrenic patients of both sexes and a normal male subject were given infusions of epinephrine labeled with carbon^{14} at the beta position or at the methyl group. Blood pressure and pulse were recorded at regular intervals during the infusion. Urine samples were collected at regular intervals prior to, and for at least thirty hours following infusion. Aliquots of each urine sample were extracted for epinephrine by the alumina adsorption method of von Euler and Hellner and bioassayed by the method of Gaddum and Lembeck; aliquots were also placed on duplicate planchets and counted in triplicate on a gas flow counter. The infusion and post-infusion urines were combined, lyophilized and extracted with a variety of organic solvents. Determinations of radioactivity, and paper chromatographic analyses were carried out on each extract.

The hemodynamic responses were found to be directly related to the rate of infusion of epinephrine, and were no longer apparent ten minutes after administration ceased. The infusion of epinephrine resulted in an increase in the urinary excretion of biologically active epinephrine which ranged from 1 to 5 per cent of the dose administered. In contrast to this, 91 ±3 per cent of the radioactivity contained in the infused beta-labeled epinephrine and 34 ±3 per cent of the radioactivity contained in the infused methyl-labeled epinephrine were excreted in the urine. The chromatograms of the chloroform, ether and butanol extracts of the lyophilized urine of subjects infused with beta-labeled epinephrine showed a major radioactive peak with an average Rf value of 0.77. Similar extracts of the lyophilized urine of patients infused with methyl-labeled epinephrine did not contain such radioactive peaks. The data are consistent with the hypothesis that in the metabolites of epinephrine the alpha and beta carbons are intact and attached to the benzene nucleus. In addition, approximately two thirds of the molecules of infused epinephrine lose their methyl groups during metabolism.

IT HAS been reported that when epinephrine is infused into human subjects, bioassay of the one-hour post-infusion urine could account for only 0.5–2.0 per cent of the material given (1, 2). The present study is an

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attempt to investigate the physiologic responses of human subjects to infused epinephrine, to correlate the urinary excretion of biologically active material with the recovery of metabolites in the urine, and to resolve and characterize these metabolites. Work with epinephrine labeled with carbon[14] at the beta position or at the methyl group was therefore undertaken (Fig. 1).

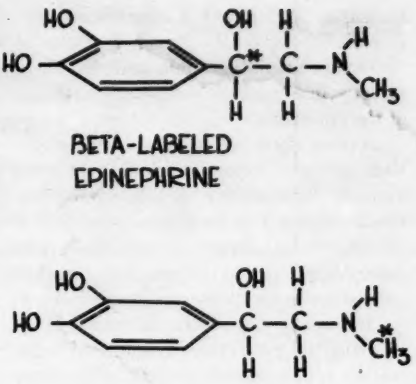

FIG. 1. Epinephrine containing isotopic carbon.

METHODS AND MATERIAL

Epinephrine. The substances infused in man were *dl*-epinephrine-7-C^{14}-*d*-bitartrate with a specific activity of 1.14 mc./mM[1] and methyl-C^{14}-*dl*-epinephrine with a specific activity of 0.16 mc./mM.[2] Both substances were shown to be homogeneous in the butanol-acetic acid-water (4:1:5) paper chromatography system. The *dl*-epinephrine-7-C^{14}-*d*-bitartrate will be referred to hereafter as β-C^{14}-*dl*-epinephrine-*d*-bitartrate.

Experimental procedure. An aliquot of the isotopically labeled epinephrine of known specific activity and weight was dissolved in sterile physiologic solution of sodium chloride for infusion into the antecubital vein. At the end of the infusion, the solution remaining in the apparatus was measured so as to determine the amount of radioactivity actually administered. All of the infusions were performed at approximately 10:00 A.M. Eleven infusions were given to schizophrenic patients of both sexes, and 1 to a normal male control. The subjects ranged in age from 41 to 57 years. They voided upon waking and were kept at rest in bed. Blood pressure and pulse readings were made at regular intervals before, during, and after the infusion. The waking urine samples were discarded and additional urine samples were collected just prior to, during, and after the infusion. The urine samples were either lyophilized or stored in the deep-freeze. The subjects, out of bed, resumed their normal daily activities fifteen to thirty minutes after the infusion.

Extraction and bioassay of epinephrine. Aliquots of urine ranging from half-hour to one-hour excretion equivalents were subjected to hydrolysis for five minutes and extracted by the alumina adsorption method of von Euler and Hellner (3). The extracts were bioassayed by a modification of the method of Gaddum and Lembeck (4), which consisted of testing the extract on the rat colon for norepinephrine and on the rat uterus for epinephrine.

Determination of radioactivity. Known volumes of urine, extracts of urine or extracts of lyophilized urine were placed on planchets and counted in triplicate on a gas-flow counter. The counts for duplicate planchets did not vary one from another by more

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[1] The *dl*-epinephrine-7-C^{14}-*d*-bitartrate was obtained from Tracerlab, Inc.

[2] The methyl-C^{14}-*dl*-epinephrine was generously furnished by Dr. Richard Schayer of the Rheumatic Fever Institute, Chicago, Illinois.

than 5 to 10 per cent. Corrections were made for the self-absorption produced by the material placed on the planchets. Strips of Whatman #1 paper 2 cm. wide were employed for paper chromatographic analysis. These strips were cut into 2-cm. squares and counted directly in a gas flow counter. Whatman #1 paper absorbs, on the average, 75 per cent of the emissions.

Method of extraction. Aliquots of whole urine, whether subjected to hydrolysis or not, were extracted twice with double their volume of a series of organic solvents. The solvent extracts were dried over anhydrous sodium sulfate and were concentrated down to a small volume in a vacuum at 45–50° C. or under nitrogen in a water-bath regulated at 45–50° C. An aliquot of each concentrate was placed on a planchet for radioactivity determinations and the remainder was used for paper chromatographic analysis. Similar extraction procedures were employed with known weights of the lyophilized urine.

Methods of paper chromatography. Paper chromatographic analysis of urine, extracts of urine, or extracts of lyophilized urine were carried out in the butanol-acetic acid-water (4:1:5) solvent system. Aliquots of each sample were placed on descending strips (2 cm. wide) of Whatman #1 paper, dried under a stream of nitrogen, and allowed to develop for approximately eighteen hours at 24° C. in the solvent system. The developed paper strips, after drying, were cut into 2-cm. squares and counted directly in a gas flow counter. When desired, the paper strips were sprayed with solutions of potassium ferricyanide, Ehrlich's reagent or ammoniacal silver nitrate. Paper chromatographic analysis was also carried out in the phenol system (95 ml. of liquified phenol, 2 ml. of water and 1 ml. of concentrated hydrochloric acid).

Methods of calculation. All of the calculations concerning cardiovascular responses and biologic activity in the urine are based upon the *l*-epinephrine equivalent of the *dl*-

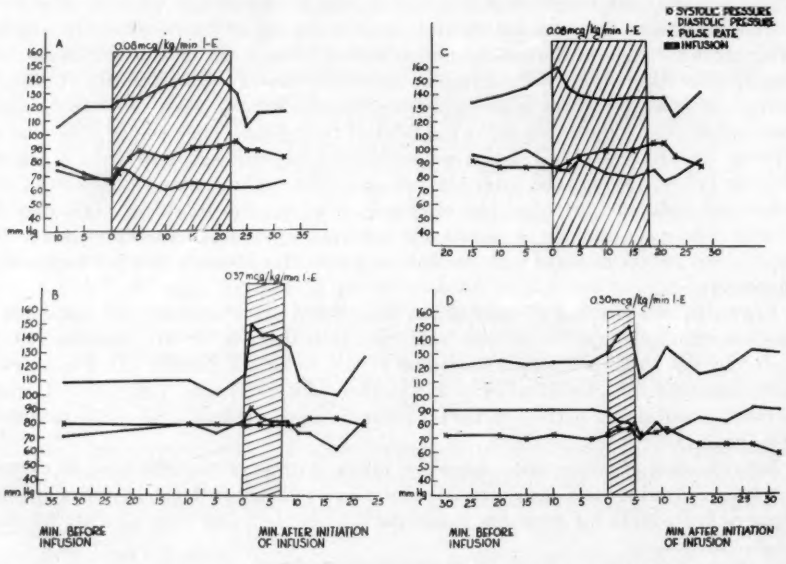

FIG. 2. Blood pressure and pulse measurements in subjects receiving infusions of epinephrine containing isotopic carbon. Ordinate—blood pressure (mm. Hg) and pulse (beats per minute). Shaded areas represent periods of infusion.

epinephrine infused, and the measurements of radioactivity and the metabolites are based upon both the *d* and *l* forms.

RESULTS

Cardiovascular responses to infusion of epinephrine

The systolic blood pressure became elevated almost immediately after the onset of the infusion, was maintained at a higher than control level during the infusion, and returned to pre-infusion levels within ten minutes after the infusion. The diastolic pressure, although variable, tended to decrease and the pulse rate became elevated during the infusion. There was a tendency for the diastolic pressure to drop after the cessation of the infusion, before returning to control levels. These observations are in agreement with those reported in the literature (1, 5). Representative cardiovascular responses to infused epinephrine are shown in Figure 2. The rate of infusion was calculated on the basis of the content of the *l*-isomer present in the epinephrine infused.

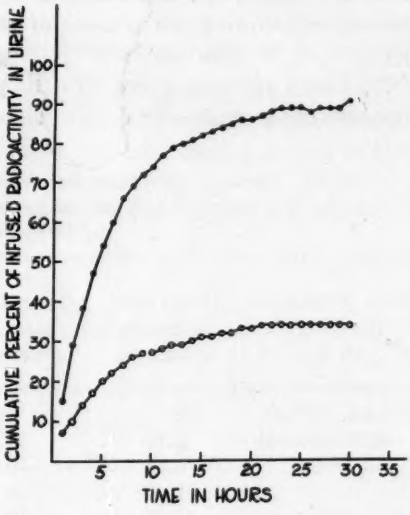

FIG. 3. The average cumulative hourly urinary excretion of total radioactivity. A solid dot represents the average value for 7 infusions with β-labeled epinephrine. An open circle represents the average value for 3 infusions with methyl-labeled epinephrine.

Urinary excretion of total radioactivity

The average cumulative hourly excretion of total radioactivity in the urine following infusion of epinephrine containing isotopic carbon is shown in Figure 3. The excretion of radioactivity in the urine was very rapid during the first few hours following the onset of the infusion. In the subjects infused with the β-C^{14}-*dl*-epinephrine-*d*-bitartrate, 91 ±3 per cent of the total radioactivity infused could be accounted for in the urine within ap-

proximately thirty hours. In the subjects infused with methyl-C^{14}-*dl*-epinephrine, 34 ±3 per cent of the total radioactivity infused could be accounted for in the urine. No additional radioactivity could be detected in the urine after thirty hours with either type of infusion. This is essentially in agreement with the work of Schayer and his group in the rat (6, 7).

Relation between urinary excretions of total radioactivity and of epinephrine

The infusion of either type of isotopically labeled epinephrine resulted in an increase in the urinary excretion of non-metabolized biologically active epinephrine. This increase was demonstrable primarily during the first few hours after the onset of the infusion, after which the urinary excretion of biologically active epinephrine tended to return to the control levels. These observations are in agreement with those reported in the literature following the infusion of non-labeled epinephrine to human subjects (1, 2, 5). The increase in these experiments ranged approximately from 1 to 5 per cent of the infused epinephrine. In contrast to this, 91 ±3 per cent of the radioactivity contained in the infused β-labeled epinephrine

TABLE 1. URINARY EXCRETION OF EPINEPHRINE AND OF RADIOACTIVITY FOLLOWING INFUSION OF LABELED EPINEPHRINE INTO AN ADRENALECTOMIZED FEMALE PATIENT

Dose of epinephrine infused (mg.)	Hours after beginning of infusion	Gamma of epinephrine in urine sample*	Cumulative % of infused *l*-epinephrine in urine sample	Cumulative % of infused radioactivity in urine sample
0.5 mg. β-C^{14}-*dl*-epinephrine-*d*-bitartrate	0	0†	0	0
	3.69	1.00	0.70	40.2
	6.44	0	0.70	67.4
	21.69	0	0.70	90.1
	25.27	0	0.70	91.4
	27.69	0	0.70	92.0
	29.44	0	0.70	92.0
1.6 mg. methyl-C^{14}-*dl*-epinephrine	0	0†	0	0
	3.25	1.88	0.24	10.9
	7.42	0	0.24	23.5
	11.42	0	0.24	26.4
	20.67	0	0.24	29.6
	24.92	0	0.24	30.0
	27.17	0	0.24	30.0
	29.17	0	0.24	30.0

* Bioassayed by the method of Gaddum and Lembeck (4).

† Pre-infusion urine sample.

and 34 ± 3 per cent of the radioactivity contained in the infused methyl-labeled epinephrine were excreted in the urine. Essentially similar results were obtained when epinephrine labeled with carbon[14] was infused into an adrenalectomized, schizophrenic female patient. These data are shown in Table 1.

The bioassays of the urine extracts measured biologically active epinephrine in the form of its *l*-isomer. The radioactivity recovered in the urine, on the other hand, was due to the metabolites derived from both the *d* and *l* isomers of the isotopically labeled epinephrine, as well as the non-metabolized fraction. As the increase in biologically active epinephrine in the urine represented on the average from 1 to 5 per cent of the infused material, the amount of non-metabolized *dl*-epinephrine would probably be twice this value.

Paper chromatographic studies of the urine following infusion of epinephrine

The chromatograms of urine obtained from patients who had received either the β-labeled or methyl-labeled epinephrine showed groups of radioactive peaks beginning close to the starting line and running almost to the solvent front.

Lyophilized urine obtained from patients receiving infusions of β-labeled

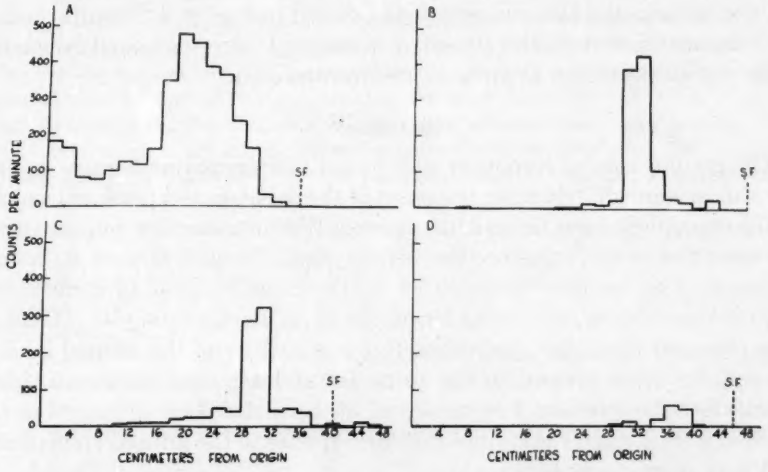

FIG. 4. Descending chromatograms of urine obtained from a patient (Cr) after the infusion of β-labeled epinephrine. Strips of Whatman #1 paper 2 cm. wide were developed in the butanol-acetic acid-water system. Ordinate—net counts per minute, uncorrected for absorption by the paper. Abscissa—centimeters from origin. S.F. = solvent front. A = acetone-alcohol; B = ether, Rf 0.67–0.75; C = butanol, Rf 0.73–0.78; D = chloroform, Rf 0.78–0.87.

epinephrine was extracted with acetone-alcohol, benzene, butanol, chloroform, and ether. These extracts were concentrated, run on paper and counted directly in a windowless flow counter, as described in the section on methods. The chromatograms of these extracts are shown in Figure 4. The chromatograms of the acetone-alcohol extracts resembled those of concentrated urine. The chromatograms of the chloroform, ether and butanol extracts showed a major peak fairly close to the solvent front. The benzene extract did not contain enough radioactivity for accurate paper chromatographic analysis. When aliquots of whole urine were extracted with benzene, butanol, ether, ethyl acetate and isopropyl acetate, a mixture of radioactive peaks of varying flow rates was removed. The chloroform and methylene-chloride extracts showed no significant radioactive peaks. Hydrolysis was then carried out on the urine which had been treated with the various solvents in the process of extraction. After hydrolysis, the urine was re-extracted with ether, chloroform and butanol. These extracts were found to contain significant amounts of radioactive material and, when chromatographed, groups of radioactive peaks of varying flow rates were observed.

Lyophilized urine of patients who had received infusions of methyl-labeled epinephrine was also extracted with acetone-alcohol, benzene, butanol, chloroform and ether. The chloroform, ether and butanol extracts did not contain the fast running peaks shown in Figure 4. Results similar to those mentioned in the foregoing paragraph were obtained when the urine was subjected to hydrolysis and re-extracted.

DISCUSSION

The cardiovascular responses to infusion of epinephrine became apparent almost immediately after the onset of the infusion and persisted as long as the epinephrine was being administered. Within a very few minutes after the cessation of the infusion, the hemodynamic responses were no longer apparent. This could indicate either a very rapid removal of epinephrine from the circulation, or a rapid formation of an inactive complex. It can be seen from our data that the radioactive metabolites of the infused labeled epinephrine were present in the urine for at least twenty-four to thirty hours after the infusion. Practically all of the metabolites appeared in the urine at a time when the cardiovascular response to the infused epinephrine had long since disappeared.

Our studies showed that infusion of epinephrine resulted in an increased urinary excretion of non-metabolized epinephrine which occurred during the first few hours after the onset of the infusion. This increased output was found to represent only a small percentage of the infused epinephrine, yet at this same period of time approximately one third of the infused β-

labeled epinephrine was recovered in the urine as radioactive metabolites. Thus the excretion of non-metabolized epinephrine is merely a minute fraction of the total amount of epinephrine infused.

Essentially all of the radioactivity contained in the infused β-labeled epinephrine could be accounted for in the urine within thirty hours following the infusion. This is presumptive evidence that the beta carbon of the epinephrine molecule remained attached to the benzene ring during metabolism. Our data also indicate that only a third of the radioactivity contained in the infused methyl-labeled epinephrine could be accounted for in the urine. Thus, one may assume that approximately two thirds of the molecules of the infused epinephrine lost their methyl groups during metabolism. Schayer *et al.* (6–8) obtained evidence to indicate that in the rat the alpha and beta carbons remain attached to the benzene ring and that cleavage of the aliphatic chain occurs somewhere between the methyl group and the alpha carbon. Epinephrine labeled with carbon^{14} in the alpha position is thus indicated for infusion in man.

Acknowledgments

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METABOLISM OF FREE AND CONJUGATED 17-HYDROXYCORTICOSTEROIDS IN SUBJECTS WITH UREMIA*

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ABSTRACT

In subjects with chronic renal disease, levels of free 17-hydroxycorticosteroids (17-OHCS) in plasma were measured at 8 A.M. and were normal. Conjugated levels were elevated, correlating inversely with endogenous creatinine clearance (Clcr.). After standard infusions of cortisol, free levels decayed exponentially but more slowly than normal, while conjugated levels rose further than in normal subjects. Conjugated, but not free, 17-OHCS again correlated inversely with Clcr. Cortisol was partially identified in the free fraction and tetrahydrocortisol and tetrahydrocortisone in the conjugated fraction of plasma following infusion. Urinary excretion of 17-OHCS and the increment after infusion were less than normal and correlated directly with Clcr. A patient with anasarca provided an exception to the noted patterns and correlations. In subjects with acute renal failure, elevated levels of conjugated 17-OHCS in plasma did not decline with the onset of diuresis but fell in parallel with levels of blood urea.

Diminished urinary excretion of 17-OHCS in chronic renal disease is attributable to failure of clearance by the damaged kidney. This results in accumulation of conjugated 17-OHCS in plasma. There appears to be correlation between degree of reduction in 17-OHCS excretion in urine, accumulation of conjugated 17-OHCS in plasma, and impairment of glomerular filtration. The observations in acute renal failure emphasize the relationship of removal of conjugated 17-OHCS from plasma to glomerular filtration rather than to urine flow. In uremia, the capacity to conjugate 17-OHCS appears to be unimpaired. Levels of conjugated 17-OHCS in plasma are lowered in the presence of anasarca by the increased volume of distribution. When the excretion of 17-OHCS in urine is used for evaluation of adrenocortical activity, careful assessment of renal function is necessary.

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INTRODUCTION

IT HAS been clearly established that there is diminished excretion of steroids in the urine of patients with renal impairment (1–3). When methods became available for the measurement of free 17-hydroxycorticosteroids (17-OHCS) in plasma (4, 5), it was noted in our laboratory that the levels of free 17-OHCS in the plasma of certain patients with renal impairment were normal although the amount of 17-OHCS excreted in the urine was greatly reduced (2). The recent development of techniques for the measurement of conjugated 17-OHCS in plasma (6) has given us an opportunity to make a more significant study of metabolism of these steroids in subjects with uremia, inasmuch as urinary 17-OHCS are excreted mainly as conjugates with glucuronic acid. This communication will report observations on the levels of free and conjugated 17-OHCS in the plasma and urine of a group of patients with renal insufficiency.

MATERIALS AND METHODS

Ten patients with chronic renal disease and impaired renal function were studied. The age and sex of each subject, and the diagnosis are indicated in Table 1. Each patient was chronically ill but none was in extremis at the time of study. One of them (V.D.) manifested the nephrotic syndrome, but all of the others were free of edema. At 8 A.M. each patient received intravenously 1 mg./Kg. of cortisol in solution over a thirty-minute period. Blood samples were drawn for estimation of free and conjugated 17-OHCS levels in the plasma at zero, half, one, two, four and six hours after the beginning of the infusion and, in most patients, at ten and twenty-four hours after the beginning of the infusion. Collections of urine (24-hour) were made during a control period and on the day of cortisol administration. Details of the standard infusion protocol and the collection and storage of specimens have been published (7). Endogenous creatinine clearances were measured in each subject. Bromsulphalein retention forty-five minutes after a dose of 5 mg./Kg. was determined in each patient and was normal in every instance.

In 1 patient with chronic renal disease and impaired renal function (D.J.), 100-ml. samples of blood were drawn two hours and twenty-four hours after the start of the standard cortisol infusion. The plasma was separated immediately and stored in a frozen state until qualitative analysis by paper chromatography could be performed. The plasma was treated in precisely the same manner as in the quantitative determination of free and conjugated 17-OHCS (4, 5, 6, 8), except that neither fraction was run through the columns. At this point the extracts containing the free and conjugated 17-OHCS were chromatographed on paper according to the method of Bush (9). Partial identification was accomplished by ultraviolet fluorescence, by reaction with blue tetrazolium, by spatial relation to known standards in the Bush system, and by elution and reaction with phenylhydrazine-sulfuric acid to determine recovery of material with an absorption maximum of 410 λ (10).

Two patients with chronic renal disease and impaired renal function (D.J. and F.R.) received intravenously 25 I.U. of adrenocorticotropic hormone (ACTH) over a six-hour period and blood samples were drawn for measurement of free and conjugated 17-OHCS levels in the plasma at zero, two, four and six hours after the start of the infusion. Details of the standard ACTH stimulation test have been published (11).

TABLE 1. EFFECT OF CORTISOL INFUSION ON PLASMA LEVELS AND URINARY EXCRETION OF 17-HYDROXYCORTICOSTEROIDS IN CHRONIC RENAL DISEASE

Patient, Age & Sex	Diagnosis	Blood urea N (mg./100 ml.)	Endogenous creatinine clearance (ml./min.)	Plasma 17-OHCS levels (μg./100 ml.)												Urinary 17-OHCS excretion mg./24 hrs.	
				Control		0.5 hr.		1 hr.		2 hrs.		4 hrs.		6 hrs.		24 hrs.			
				F*	C†	F	C	F	C	F	C	F	C	F	C	F	C	Control	Infusion
V.D., 36, M‡	Nephrosis; chronic glomerulonephritis	90	9	13	20	144	43	91	49	59	60	37	60	23	60	17	45	2.4	6.2
J.G., 48, M	Chronic glomerulonephritis	112	5	11				88		58		39		24				0.4	1.3
J.G., 48, M	Chronic glomerulonephritis			14	110	110	138	66	143	55	146	39	151	27	142	14	141		3.5
J.W., 58, M	Chronic pyelonephritis	92		14				82		62		36		24				1.1	2.3
R.J., 34, M	Chronic glomerulonephritis; adrenal hyperplasia	116	4	19	133	134	163	89	173	68	217	38	184	25	183	14	196	0.9	7.8
F.C., 72, M	Chronic pyelonephritis	90	10	16	85	128	97	91	108	68	123	40	154	22	154	13	95	0.5	4.7
D.J., 40, M	Polycystic kidney disease	92	7	10	62	101	78	79	96	57	107	33	113	22	101	11	101	0.0	2.1
			Mean	*14*	*98*	*118*	*119*	*83*	*130*	*61*	*148*	*38*	*150*	*24*	*145*	*13*	*133*	*0.6*	*3.6*
			±*S.E.M.*	*1.3*	*15*	*7.7*	*19*	*3.8*	*17*	*2.3*	*24*	*1.1*	*15*	*0.8*	*17*	*0.7*	23	*0.2*	*1.0*
J.P., 22, M	Chronic pyelonephrits	47	33	17	14	102	39	67	58	49	68	26	86	19	82	8	52	1.5	9.7
D.B., 60, M	Bilateral hydronephrosis	66	25	18	33	130	47	76	66	65	74	49	89	28	85	13	18	0.8	3.4
I.D., 68, M	Chronic pyelonephritis; prostatic obstruction	28	34	14	23	99	43	76	79	49	98	25	93	15	62	9	29	2.2	4.2
F.R., 64, M	Diabetic & hypertensive nephropathy	24	57	20	11	166	31	151	80	71	81	49	90	29	85				
			Mean	*17*	*20*	*124*	*40*	*93*	*71*	*59*	*80*	*37*	*90*	*23*	*79*	*10*	*33*	1.5	*5.8*
			±*S.E.M.*	*1.3*	*4.9*	*16*	*3.4*	*2.0*	*5.3*	*5.6*	*6.5*	*6.8*	*1.4*	*3.4*	*5.5*	*1.5*	*10*	*0.4*	*2.0*

* Free.
† =Conjugated.
‡ =Levels in this patient are not included in the calculation of the mean, since the presence of anasarca altered results (see discussion).

During the courses of 2 patients with acute renal failure, serial determinations were made of the free and conjugated 17-OHCS levels in the plasma, the 24-hour urine volume and the blood urea nitrogen concentration. In one of these subjects, the acute renal failure was the result of severe burns, and in the other it was due to protracted shock following hematemesis. The occurrence of severe hyperkaliemia in D.G.G. necessitated a six-hour period of hemodialysis with the Skeggs-Leonard artificial kidney. The conjugated 17-OHCS content of the dialyzing fluid was determined. The levels of free and conjugated 17-OHCS in the plasma of a third patient with complete ureteral obstruction were also determined.

The estimation of free 17-OHCS in the plasma was performed in accordance with the modified technique of Nelson, Samuels and Eik-Nes (4, 5). With this method, results are reported in terms of a cortisol standard. Conjugated 17-OHCS in plasma were measured by a modification (8) of the method of Bongiovanni (6), in which the conjugated 17-OHCS are hydrolyzed with β-glucuronidase and the resultant free 17-OHCS are determined after dialysis. With this technique, results are reported in terms of a cortisone standard. The free and conjugated 17-OHCS levels in the urine were estimated by a modification (7) of the method of Reddy, Jenkins and Thorn (12), in which results are reported in terms of a cortisone standard. These values were added together to estimate total 17-OHCS excretion in the urine. It should be noted that these techniques measure the 17,21-dihydroxy,20-ketosteroids, which include cortisol, cortisone, Reichstein's substance S and their hydrogenated derivatives. In the normal subject, cortisol is the chief constituent of the free 17-OHCS in the plasma (13), whereas hydrogenated derivatives of cortisol and cortisone make up the conjugated fraction (14). In urine, tetrahydrocortisone and tetrahydrocortisol glucuronides are the principal 17-OHCS (15, 16).

RESULTS

Control levels of free and conjugated 17-OHCS in plasma in chronic renal disease

The 8 A.M. levels (means and standard errors of the mean) of free and conjugated 17-OHCS in the plasma in a series of normal subjects were 12.0 ± 0.7 μg. per 100 ml. and 12.0 ± 1.6 μg. per 100 ml., respectively (8). The corresponding values in patients with renal disease are shown in Table 1. It may be seen from this table that the plasma levels of free 17-OHCS were in the upper part of the normal range, 10–20 μg. per 100 ml. The plasma levels of conjugated 17-OHCS were elevated in all but 1 instance (F.R.). The patients could be separated into two groups: 1) those with clinically severe impairment of renal function and endogenous creatinine clearances below 11 ml. per minute, and 2) those with clinically moderate impairment of renal function and endogenous creatinine clearances between 25 and 60 ml. per minute. The mean plasma conjugated 17-OHCS level in the first group (98 μg. per 100 ml.) was significantly increased above normal ($P < 0.001$), whereas in the second group it was not elevated significantly (20 μg. per 100 ml.). One patient with the nephrotic syndrome in addition to severe renal functional impairment (V.D.) did not manifest the elevated levels seen in the first group.

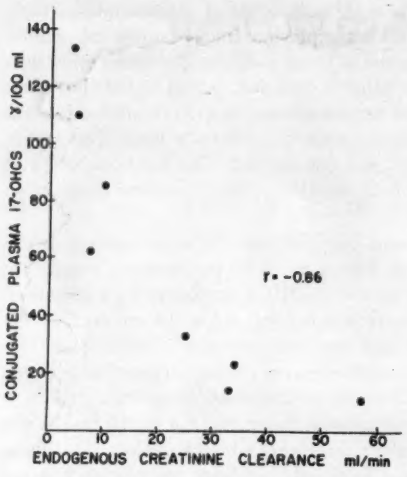

FIG. 1. Scatter diagram depicting correlation between endogenous creatinine clearance and 8 A.M. levels of conjugated 17-OHCS in the plasma of patients with uremia.

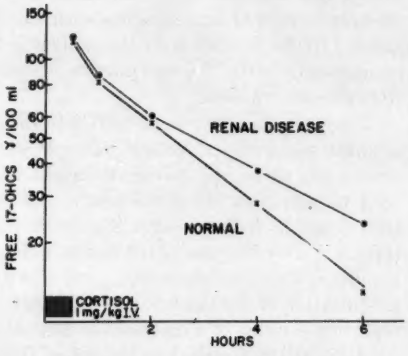

FIG. 2. Mean levels of free 17-OHCS in the plasma after an infusion of cortisol in patients with uremia and in normal subjects.

When the individual plasma levels of conjugated 17-OHCS were plotted against the endogenous creatinine clearances of these subjects (Fig. 1), an inverse correlation was observed ($r = -0.86$).

Plasma free 17-OHCS levels after the infusion of cortisol

The levels of free 17-OHCS in the plasma after administration of cortisol to patients with chronic renal disease are shown in Table 1. In normal subjects (8) the levels of free 17-OHCS after the infusion of cortisol fell rapidly until one hour after the beginning of the infusion. Thereafter they diminished in an exponential fashion to approach the normal levels of diurnal variation six hours after the beginning of the infusion (Fig 2). The mean slope of the curve of removal of free 17-OHCS from the plasma was $-0.162 \pm$S.E.M. 0.007, and the half-life of the administered cortisol was $112 \pm$S.E.M. 5 min.

The levels of free 17-OHCS in the plasma of patients with impaired renal function after infusion of cortisol followed the same pattern as those in the normal subjects. However, the plasma levels in these patients did not decline in a logarithmic manner with time until a point between one and two hours after starting the administration of cortisol. Furthermore, the rate of removal of free 17-OHCS from the plasma was slower than it was in the normal subjects (Fig. 2). The mean slope of removal from the plasma in all patients with uremia was -0.110 ± 0.007 (half-life = 164

min.), which was significantly different from that in the normal subjects ($P < 0.01$). The rate of removal of free 17-OHCS from the plasma of the subjects with renal insufficiency was not significantly related to the degree of renal insufficiency as estimated clinically or by endogenous creatinine clearance ($P = 0.6$). In subjects with creatinine clearances below 11 ml. per minute the mean slope of the removal curve was -0.106 ± 0.004 (half-life = 172 min.), and in those with creatinine clearances between 25 and 60 ml. per minute it was -0.117 ± 0.018 (half-life = 155 min.).

Plasma conjugated 17-OHCS levels after administration of cortisol

The administration of cortisol to normal subjects resulted in a rise of conjugated 17-OHCS in plasma to a mean maximum level of $53 \pm$ S.E.M. 2.3 μg. per 100 ml. two hours after the onset of the infusion (8). Following this, the levels declined exponentially to approach control values ten hours after the onset of the infusion (Fig. 3). The plasma levels of conjugated 17-OHCS in the subjects with impaired renal function once again reflected the degree of functional derangement. The mean levels of conjugated 17-OHCS in the patients with severe renal impairment rose to a peak of 150 ± 15 μg. per 100 ml. two hours after beginning the infusion and remained at approximately this level throughout the 24-hour period of observation. Specimens for analysis of conjugated 17-OHCS levels in the plasma were not obtained after the 24-hour period but, by extrapolation, it appeared that approximately three days would be required for a return to control values. In the patients with less severe renal decompensation, the mean conjugated 17-OHCS levels in the plasma were 80 ± 6.5 μg. per 100 ml. and 90 ± 1.4 μg. per 100 ml. at two and four hours, respectively, after the beginning of the infusion. Thereafter these levels declined slowly to approach control values twenty-four hours after the infusion (Table 1). The levels of conjugated 17-OHCS in the plasma of V.D., the subject with severe renal impairment

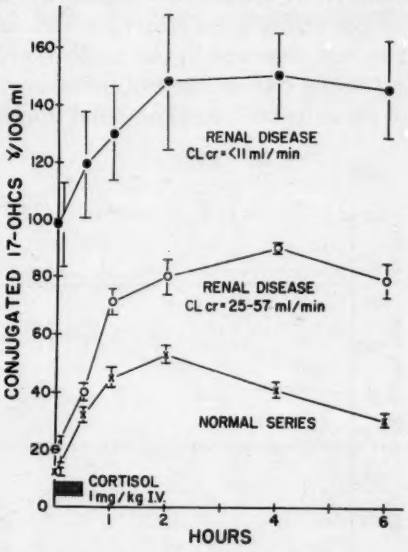

FIG. 3. Mean levels of conjugated 17-OHCS in the plasma after an infusion of 1 mg./Kg. of cortisol in patients with advanced renal insufficiency, in patients with early uremia and in normal subjects. The height of pre-infusion levels and the post-cortisol peaks are correlated with the degree of renal impairment.

and the nephrotic syndrome, were considerably lower than those in the patients with a comparable degree of renal impairment.

Analysis of plasma 17-OHCS by paper chromatography

In the specimens drawn at two and at twenty-four hours after the infusion, cortisol was the only 17-OHCS in the free fraction of the plasma, but tetrahydrocortisol and tetrahydrocortisone were present in the conjugated fraction.

Urinary excretion of 17-OHCS in chronic renal disease

The mean 24-hour excretion of total 17-OHCS in the urine of subjects with severe degrees of renal impairment was $0.6 \pm$ S.E.M. 0.2 mg., that of patients with lesser degrees of renal functional derangement was 1.5 ± 0.4 mg., and that of subjects with normal renal function (7) was 5.8 ± 1.2 mg. (Table 1). There was good correlation between the urinary excretion of total 17-OHCS and the degree of renal functional impairment as estimated by the endogenous creatinine clearance ($r = 0.82$, Fig. 4). Such correlation was not observed in the patient with the nephrotic syndrome (V.D.).

On the day of cortisol infusion, the mean urinary 17-OHCS excretion of patients with marked renal impairment was 3.6 mg. (range 1.3 to 7.8), that of patients with less renal impairment was 5.8 mg. (range 3.4 to 9.7), and that of normal subjects (7) was 18.4 mg. (range 11.1 to 24.9) (Table 1).

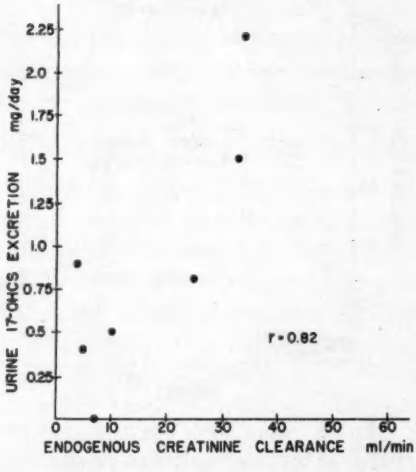

FIG. 4. Scatter diagram showing the relationship of endogenous creatinine clearance and excretion of total 17-OHCS in the urine of patients with uremia.

Plasma levels of free and conjugated 17-OHCS after stimulation with ACTH

Plasma levels of free 17-OHCS in D.J., a subject with severe impairment of renal function, at zero, two, four and six hours after beginning the infusion of 25 I.U. of ACTH were 8, 17, 19 and 26 μg. per 100 ml., respectively. The levels in F.R., a patient with less severe renal functional impairment, were 19, 37, 40 and 40 μg. per 100 ml., respectively, at these times. Such responses of plasma free 17-OHCS levels to ACTH stimulation are in the lower range of normal (8, 11). The levels of conjugated 17-OHCS in the plasma of

D.J. were 112, 116, 121, 124, 147, 143 and 111 μg. per 100 ml. at zero, two, four, six, ten, fifteen and twenty-four hours, respectively, after the beginning of the six-hour infusion.

Plasma levels of free and conjugated 17-OHCS in acute renal failure

Serial observations of plasma free and conjugated 17-OHCS levels, daily volume of urine and blood urea nitrogen level were made in 2 patients with acute renal failure, both of whom were severely ill. The values for free 17-OHCS ranged from normal to 40 μg. per 100 ml. in D.G.G. (Fig. 5), but they were not elevated in J.D.P. (Fig. 6). At no time was there a correlation between free 17-OHCS levels and the other parameters studied. The levels of conjugated 17-OHCS in the plasma of these subjects were strikingly elevated during the entire period of oliguria and did not decline with the onset of diuresis. The maximum level attained in D.G.G. was 334 μg. per 100 ml., and in G.D.P. 188 μg. per 100 ml. The relation of conjugated 17-OHCS levels in plasma to urinary volume and blood urea nitrogen concentration is depicted in Figures 5 and 6.

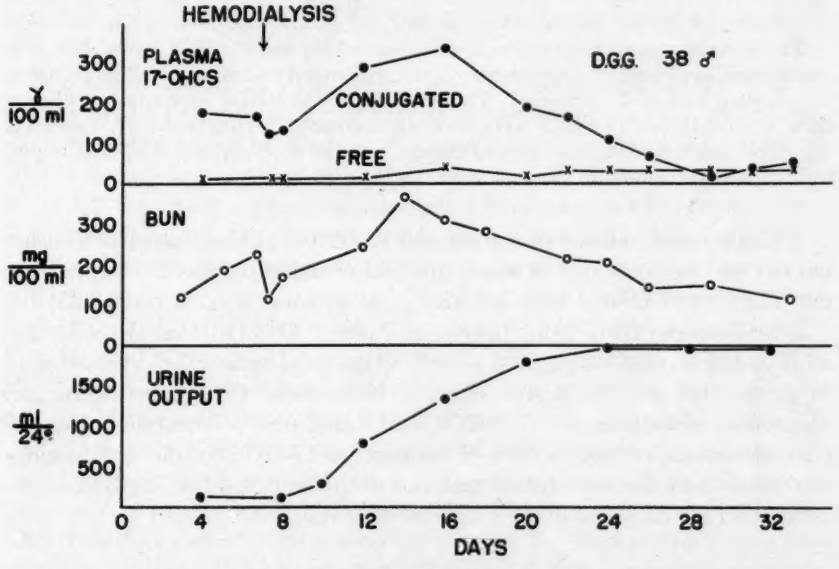

FIG. 5. Serial observations of plasma free and conjugated 17-OHCS levels, daily output of urine and blood urea nitrogen level in a patient with acute renal failure following extensive burns. Note the transient decline in the level of plasma conjugated 17-OHCS during dialysis with the artificial kidney and the striking parallelism of the declining levels of conjugated 17-OHCS and blood urea nitrogen during recovery of renal function.

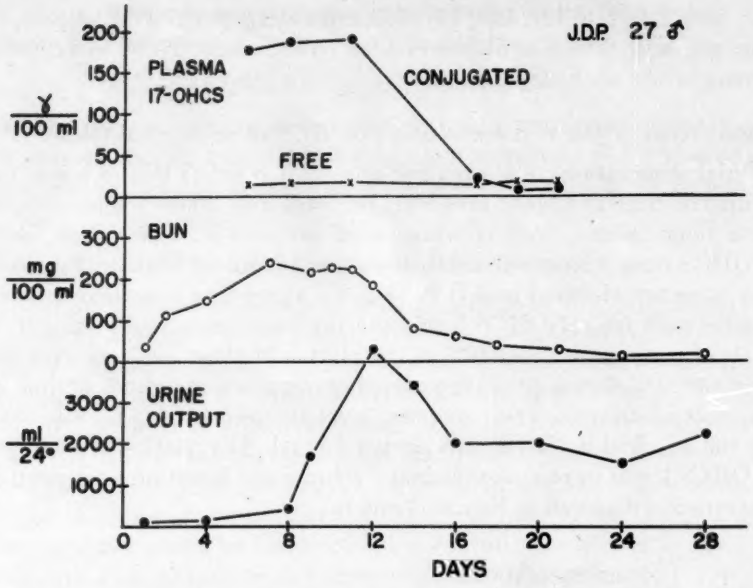

FIG. 6. Serial observations of free and conjugated plasma 17-OHCS levels, daily output of urine and blood urea nitrogen level in a patient with acute renal failure following hematemesis and prolonged shock. The levels of free 17-OHCS were normal, whereas those of conjugated 17-OHCS were elevated markedly. During recovery, increasing urine flow was unaccompanied by any change in the level of conjugated 17-OHCS until the clearance of blood urea nitrogen began to improve.

A single measurement of conjugated 17-OHCS in the plasma of another patient on the ninth day of acute ureteral obstruction was 284 μg. per 100 ml. The free 17-OHCS level in this specimen was 19 μg. per 100 ml.

A six-hour period of hemodialysis in Patient D.G.G. (Fig. 5) resulted in a fall in the level of conjugated 17-OHCS in the plasma which amounted to 37 μg. per 100 ml. The dialysate from the artificial kidney contained 15.5 milligrams of conjugated 17-OHCS. Assuming free permeability, the calculated volume of distribution of conjugated 17-OHCS sufficient to allow the removal of this amount of material with the noted fall in plasma concentration would be 42 liters in this 80-Kg. man.

DISCUSSION

It is apparent from the studies reported here that the diminished excretion of 17-OHCS in the urine of subjects with renal impairment is attributable to failure of the damaged kidney to clear the conjugated 17-OHCS from the plasma. As a result, these steroids accumulate in the plasma. The

close correlation of endogenous creatinine clearance with 17-OHCS excretion in the urine and the inverse correlation with the accumulation of conjugated 17-OHCS in the plasma strongly suggest that conjugated 17-OHCS are excreted, in the main, by a process of glomerular filtration. Similar conclusions have been reached by other workers. Marks and Leaf (17) found a close correlation between creatinine clearance and 17-OHCS excretion in dogs in which the glomerular filtration rate of an explanted kidney was varied by arterial constriction. Bongiovanni and Eberlein (18) noted a close correlation between endogenous creatinine clearance and the clearance of conjugated 17-OHCS in normal subjects given large oral doses of tetrahydrocortisone monoglucuronide. Daughaday and Bremer (19) also raised the possibility of tubular secretion as an additional excretory mechanism.

In the foregoing discussion, the terms total and conjugated have been used interchangeably in referring to 17-OHCS levels in the urine. Actually, the conjugated fraction of the 17-OHCS in the urine constitutes about 95 per cent of the total. This has been established in normal subjects (7), in patients with liver disease (7) and in the present study. The stronger binding of plasma protein to free 17-OHCS than to conjugated 17-OHCS has been invoked to explain the negligible renal clearance of the free material (19).

It is noteworthy that the elevated levels of conjugated 17-OHCS in the plasma of the patients with acute renal failure (Figs. 5 and 6) did not decline with the onset of diuresis. However, the levels of conjugated 17-OHCS in the plasma did parallel the concentration of blood urea nitrogen, a constituent which is excreted principally by glomerular filtration.

The explanation for the subnormal rate of removal of free 17-OHCS from the plasma of patients with uremia is a matter for conjecture. Accumulation in the plasma of the small fraction of free 17-OHCS which is normally excreted in the urine would not account completely for these results. Furthermore, there was no relationship between the rate of removal of free 17-OHCS and the degree of renal impairment in the small group of patients studied. Of the factors which slow the rate of removal of free 17-OHCS from the plasma—namely, impaired liver function (7), old age (20) and decreased basal metabolic rate (21)—only grossly impaired hepatic function has been excluded. The mean age of the subjects with impaired renal function was 50 years, whereas that of the subjects in the normal series was 34 years. The basal metabolic rate (BMR) was not measured in the uremic subjects. Although the reported BMR's in patients with nephritis are variable (22), a low BMR might be expected in our patients because their protein intake was low (23) and they were largely in the older age group (24). It is also possible that the accumulation of conjugated 17-

OHCS in the plasma may have decreased the rate of removal of the free 17-OHCS by mass action.

The response of the levels of free 17-OHCS in the plasma to ACTH stimulation of the adrenal cortex in 2 subjects with impaired renal function was in the lower part of the range observed in normal individuals.

It is of interest that the conjugating capacity of patients with poor renal function was not seriously impaired after administration of cortisol. The degree and duration of the elevation of conjugated 17-OHCS levels in the plasma were proportional to the reduction of glomerular filtration. This would suggest that there is little if any participation by the kidney in the process of conjugation of 17-OHCS.

The results of analysis by partition chromatography of the free and conjugated 17-OHCS in the plasma of a patient with uremia were similar to those reported in normal subjects (14). Thus, there is no evidence that impaired renal function changes the normal route of metabolism of 17-OHCS. Additional qualitative analyses of cortisol metabolism were not performed. Peterson and associates (16) have shown that, in normal subjects, 4 per cent of a dose of labeled cortisol appeared in the urine as free 17-OHCS, 20–30 per cent as 17-OHCS glucuronide, 30 per cent as non-phenylhydrazine-reacting glucuronides and 10–20 per cent as non-glucuronide conjugates. However, in the present study, only phenylhydrazine-reacting steroids were measured.

The level of conjugated 17-OHCS in the plasma may be misleading in subjects with fluid retention. The level in the patient who had the nephrotic syndrome with advanced renal insufficiency was but slightly elevated and, after the infusion of cortisol, the increment was much less than that in other patients with uremia. The low levels in this patient reflected the increased volume of distribution of conjugated 17-OHCS rather than an impairment of 17-OHCS conjugating capacity. Similar observations have been made in patients with cirrhosis of the liver and ascites (25).

The decreased excretion of 17-OHCS in the urine of patients with impaired renal function illustrates again the hazard of drawing conclusions concerning adrenocortical function on the basis of a single test (26). Many of the subjects in this report excreted amounts of 17-OHCS in the urine comparable to those observed in adrenocortical insufficiency even after stimulation with ACTH. We have also seen patients with Cushing's syndrome and advanced renal disease in whom the excretion of 17-OHCS in the urine was low or normal, despite elevated 17-OHCS levels in the plasma and exaggerated responses of these levels to ACTH. In these subjects the obvious hypercorticism was not detected by measurements of the urinary 17-OHCS, because of the marked impairment of renal function. Thus the excretion of 17-OHCS in the urine cannot be used as a measure of adrenocortical function without consideration of renal function.

Acknowledgment

We wish to acknowledge the assistance of Mrs. Anne Keller for the paper chromatography study, the donation of parenteral cortisol by Merck, Sharp & Dohme, the donation of ACTH by the Armour Laboratories, and the helpful advice of Dr. L. T. Samuels and Dr. M. M. Wintrobe.

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PLASMA 17-HYDROXYCORTICOSTEROID LEVELS IN PATIENTS WITH ABNORMAL GLUCOSE TOLERANCE DURING PREGNANCY*

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ABSTRACT

A study has been made of plasma 17-hydroxycorticosteroid (17-OHCS) levels in 120 women during pregnancy, at delivery, and postpartum, including analyses on umbilical cord blood. In 82 there was abnormal tolerance to oral glucose during pregnancy, but there were no clinical manifestations of diabetes mellitus and these women were not known to have been diabetic previously. Forty-one of them were treated with 15 units of NPH insulin daily. No difference in plasma levels of 17-OHCS was found between the controls, the patients with abnormal glucose tolerance who were untreated, and those with abnormal glucose tolerance who were treated with insulin, at any of the various stages in which assays were made, before and after delivery. It was concluded that there is no relationship between plasma levels of 17-OHCS and the presence of abnormal glucose tolerance during pregnancy, and that insulin has no effect on plasma 17-OHCS concentration either in the mother's blood or in the umbilical cord blood at delivery. The alterations in plasma levels of 17-OHCS in pregnancy, previously noted in the literature, are confirmed.

PREGNANCY is one of the most common conditions, other than diabetes mellitus, in which diminished glucose tolerance may be observed. In some apparently normal women, when pregnant, there is a decreased tolerance to oral glucose which spontaneously reverts to normal postpartum. In others, an abnormal tolerance to glucose during pregnancy may be the first evidence of incipient diabetes mellitus.

It is known that the administration of adrenocorticotropic hormone may elicit an abnormal response to the glucose tolerance test though a normal response has been previously obtained (1). The infusion of cortisol has

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produced a similar response in normal subjects—a decreased tolerance to glucose which imitates that seen in diabetes (2).

Plasma 17-hydroxycorticosteroid (17-OHCS) concentration rises in pregnancy, and there is a gradual return to normal postpartum (3, 4). Hoet has suggested that the development of abnormal glucose tolerance during pregnancy may depend upon the increased secretion of glucocorticoids (5). He found appreciable quantitites of corticoids in the amniotic fluid of a diabetic woman, but none in normal amniotic fluid (6).

The purpose of this study was to compare the blood concentration of 17-hydroxycorticosteroids in patients with abnormal glucose tolerance during pregnancy with the blood concentration in normal pregnant patients, and to examine the effect of insulin therapy in the former group.

MATERIALS AND METHODS

Analyses were made of the blood of 120 patients, 83 of whom had abnormal glucose tolerance during pregnancy without a previous history of diabetes mellitus. Patients were selected at random from those in a study conducted by the U. S. Public Health Service at the Boston City Hospital and the Boston-Lying-in Hospital.

Glucose determinations on blood plasma were made by the Somogyi-Nelson method (7, 8) after the patient had fasted overnight, and at one, two and three hours following the oral ingestion of 100 Gm. of glucose. The diet during the three previous days had contained at least 300 Gm. of carbohydrate. The upper limits of normal for blood glucose concentration were arbitrarily set at 110 mg. per 100 ml. fasting, 170 mg. at one hour, 120 mg. at two hours, and 110 mg. at three hours. Results of the test were considered abnormal if the concentration was above these limits in 2 or more of the analyses. The 83 patients with abnormal glucose tolerance in our study were divided into two groups, one of which was given insulin. The dosage was 15 units of NPH insulin daily. A third group with normal glucose tolerance was used for comparison. All groups were under the same prenatal supervision.[1]

The blood samples were drawn at the patient's regular clinic visit, and the delivery and cord samples were drawn at delivery. The postpartum blood specimens were obtained on the second or third day after delivery. Heparinized plasma or serum was separated and used for the analyses.

The analyses for plasma 17-OHCS were made by the method of Nelson and Samuels (9) using crystalline 17-hydroxycorticosterone as a standard.[2] Ethanol was used as the solvent in the color reaction (10).

RESULTS

The recovery of pure 17-hydroxycorticosterone dissolved in water and carried through the whole analysis is shown in Table 1. At low concentrations, the recovery was poor, but each analytical value was corrected to a theoretically complete recovery by using a standard which had also been

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[1] These determinations were made as part of the prenatal metabolic clinic study and will be reported in full elsewhere (11).

[2] Graciously supplied by Merck & Co.

TABLE 1. RECOVERY OF 17-HYDROXYCORTICOSTERONE

Amount added (μg.)	Mean amount recovered (μg.)	Percent recovery
3	2.0 (36)†	68 ±4*
6	4.4 (36)	73 ±3
9	7.3 (35)	81 ±3
12	9.6 (36)	80 ±3

* Mean and standard error of the mean.

† Figures in parentheses denote the number of determinations.

carried through the entire procedure. In blood from normal men and women, a comparison was made between the 17-OHCS content of serum and that of plasma. As shown in Table 2, there was no difference.

The serum concentrations of 17-OHCS in patients with and without abnormal glucose tolerance during pregnancy, both untreated and treated with insulin, are shown in Table 3. The small difference among the means of the groups in the second trimester is not significant. The third trimester means of 23 μg., 22 μg. and 21 μg. per 100 ml. respectively for the three groups were almost identical, and this similarity can be seen also between the means in the umbilical cord and postpartum columns. There was a considerable difference between the mean plasma 17-OHCS level of 49 μg. per 100 ml. in the abnormal glucose tolerance group and 39 μg. per 100 ml. in a similar group treated with insulin, but the wide variation among the individual results renders this difference statistically nonsignificant. Moreover, upon comparison of the plasma 17-OHCS levels at delivery with those in the third trimester in the same patients (16 insulin-treated, and 14 not insulin-treated), the means of the increased concentrations at delivery

TABLE 2. COMPARISON OF PLASMA AND SERUM 17-HYDROXYCORTICOSTEROID LEVELS IN NORMAL MALES AND FEMALES

Patient	Serum 17-OHCS (μg./100 ml.)	Plasma 17-OHCS (μg./100 ml.)
B.L.	13.2	13.8
P.C.	5.8	5.9
C.R.	9.4	9.4
C.R.	7.8	7.8
B.L.	13.4	13.1

TABLE 3. PLASMA 17-HYDROXYCORTICOSTEROID LEVELS DURING PREGNANCY (μG. PER 100 ML.)

Clinical status	2nd trimester	3rd trimester	Delivery	Umbilical cord	Post partum
Normal pregnancy	16 ±3.6* (9)†	23 ±2.2 (35)	42 ±7.2 (11)	17 ±4.5 (11)	20 ±2.6 (11)
Abnormal glucose tolerance	25 ±6.3 (7)	22 ±2.7 (33)	49 ±6.9 (17)	17 ±5.6 (6)	18 ±2.9 (12)
Abnormal glucose tolerance; treated with insulin	19 ±4.6 (5)	21 ±2.1 (42)	39 ±2.3 (17)	15 ±4.2 (7)	19 ±2.8 (11)
Combined averages	*20*	*22*	*43*	*16*	*19*

* Mean and standard error of the mean.
† Figures in parentheses denote number of cases.

were almost equal (22 μg. and 25 μg. per 100 ml.). This confirms the lack of difference between the groups as a whole.

In each group, there were significant differences between the plasma levels of 17-OHCS in the third trimester (22 μg. per 100 ml.) and at delivery (43 μg. per 100 ml.) ($P < 0.01$). There were also significant differences between the combined averages for plasma 17-OHCS at delivery (43 μg. per 100 ml.), in umbilical cord blood (16 μg. per 100 ml.) and post partum (19 μg. per 100 ml.) ($P < 0.001$) (12).

DISCUSSION

These determinations of free 17-OHCS in plasma ignore the presence of conjugated components and the influence of pregnancy on the rate of degradation and conjugation of free steroid. However, the results confirm the work of others (3, 4) in demonstrating elevated concentrations of plasma 17-OHCS in pregnancy, the sharp increase at delivery, and the lower umbilical cord and postpartum values.

There is apparently no relation between plasma 17-OHCS concentration and the presence of abnormal glucose tolerance during pregnancy in patients without previously known diabetes mellitus. Insulin had no effect on these concentrations. Thus it would appear that Hoet's hypothesis, if expressed as a causal relationship between elevated plasma levels of 17-OHCS and abnormal tolerance to oral glucose during pregnancy, is incorrect.

Determinations on plasma from umbilical cord blood are not direct

measurements of 17-OHCS concentrations in the fetus, but in part reflect the maternal concentrations. The maternal levels are unaltered by the presence of abnormal glucose tolerance, and there is no difference between the cord values in the three groups of pregnant women (Table 3). Thus the fetal umbilical-cord concentrations of 17-OHCS are unaffected by the presence of abnormal glucose tolerance in the mother, whether untreated or treated with insulin.

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OXYTOCIN IN LACTATING AND NONLACTATING WOMEN

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ABSTRACT

Blood was collected from lactating and nonlactating women with babies 3–7 months old and tested for oxytocin. A modification of the extraction method of Bisset and Walker (1954) was used. As K^+ or HT (5-hydroxytryptamine) in the extracts might be interfering substances, they were removed by dialysis or by adding Dibenamine hydrochloride to the organ bath. The extracts were assayed on the isolated sensitized rat uterus, and oxytocin and another oxytocic substance (O.S.) were differentiated by treatment of the extracts with sodium thioglycollate.

The oxytocic activity of the mother's blood collected immediately prior to breast-feeding the infant did not differ significantly from that during suckling or midway between 4-hourly feedings: moreover, it did not differ significantly from that in the blood of nonlactating mothers.

The conclusion is drawn that, in these studies on human subjects, neither oxytocin nor O.S. induces the ejection of milk.

IT HAS been postulated that during milking afferent impulses pass from receptors in the mammary gland to hypothalamic nuclei, to initiate the release into the blood stream of oxytocin from the hypothalamus-pars nervosa region and thus facilitate the expression of milk (1–3).

Recently it has been shown in women with babies 4–7 days old that the oxytocic activity (O.A.) of the mother's blood generally increases during suckling and that this is due mainly to an oxytocic substance (O.S.) which is not considered to be oxytocin (4).

In experiments on cows, when blood samples were taken at the accustomed time and place for milking, it was found that the total O.A. and the level of oxytocin in the blood were the same immediately prior to milking and during milking, suggesting a conditioned release of oxytocin before milking. Under similar circumstances goats had the same O.A. immediately before and during milking, but in them the oxytocin level rose during milking. In both cows and goats there was virtually no oxytocin in the blood three hours after milking when the animals were away from the milking pails; thus the O.A. was accounted for almost entirely by the presence of O.S. (5).

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We were interested to see whether there is a conditioned release of oxytocin in women who are breast feeding their babies at regular intervals, and also whether any difference exists between the O.A. of blood samples taken from these women and from mothers who are artificially feeding their infants because of failure of lactation. This paper records the results of the estimation of oxytocic activity and the level of oxytocic substance in blood taken from women: (a) five to ten minutes before the baby suckled; (b) during suckling, and (c) two hours after suckling (*i.e.*, mid-way between feedings). These results are compared with results in women who failed to lactate.

METHOD

Preparation of the extracts. Samples of venous blood were collected from 6 women a few minutes before the babies were due to be fed, and again after the infants had been suckling for two or three minutes. Similar samples were taken from 5 mothers at the mid-feeding interval and from 6 mothers in whom lactation failed to become established.

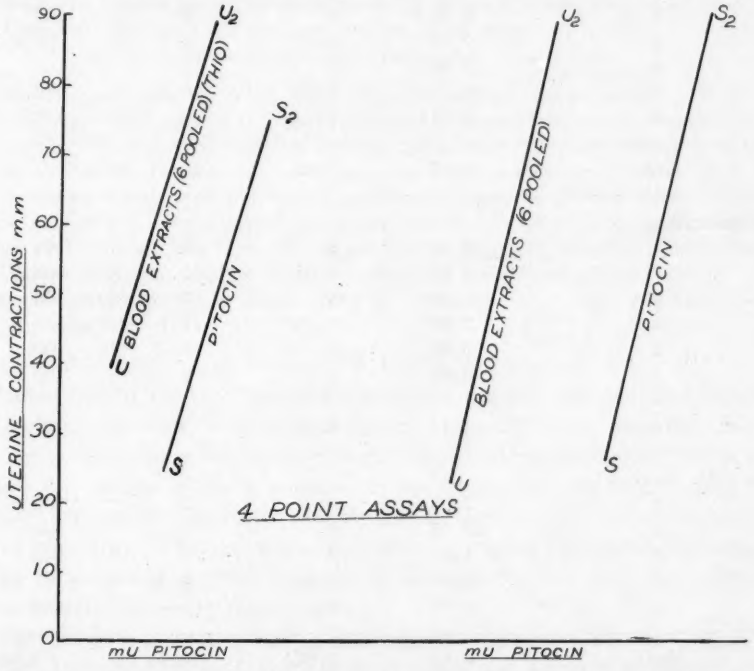

FIG. 1. Close parallelism between oxytocin and the oxytocic substance in the extracts of blood from 6 nonlactating mothers. Abscissa: Log dose in milliunits of oxytocin (Pitocin). Ordinate: Uterine contraction in millimeters. S and S_2 are the responses for the low and high doses of standard, respectively, and U and U_2 are the responses for the low and high doses of unknown, respectively.

Each sample was mixed separately with 9 volumes of 80 per cent (v/v) ethanol and *N* H_2SO_4 was added until the solution was blue to Congo Red. The extracts were then prepared by a method previously described (6).

Method of assay. Extracts were assayed for O.A., oxytocin, and O.S, against Pitocin (Parke, Davis & Co.) on the isolated uterus of virgin Wistar-strain rats in estrus, using

TABLE 1. OXYTOCIC ACTIVITY OF ACID-ALCOHOL EXTRACT OF BLOOD OF LACTATING WOMEN—INCLUDING STATISTICAL RESULTS

Number	Total oxytocic activity	Oxytocin*	% Oxytocin / Total activity	% 2nd Oxytocic substance / Total activity
	mu/ml. blood			
5–10 min. before suckling				
1	1.1	0	0	100
2	0.52	0	0	100
3	0.55	0.02	3.6	96.4
4	2.40	0	0	100
5	0.76	0.08	10.5	89.5
6	8.05	0	0	100
Mean	2.23	0.02	2.35	97.65
During suckling				
1	0.42	0	0	100
2	0.66	0.01	1.5	98.5
3	2.26	0	0	100
4	2.35	0.26	11.1	88.9
5	0.62	0	0	100
6	0.81	0	0	100
Mean	1.19	0.05	2.1	97.9
2 hrs. after suckling†				
1	7.26	0	0	100
2	23.85	0	0	100
3	1.01	0	0	100
4	0.97	0.02	2.06	97.94
5	1.15	0	0	100
Mean	6.85	0.004	0.41	99.59

* The extracts were dialyzed and the oxytocin values are uncorrected for loss through the dialysis membrane, where the mean loss of oxytocin is 50 per cent.

† Midway between four-hourly feedings.

(TABLE 1—*continued on facing page*)

Total oxytocic activity of blood in mu/ml. (oxytocin)	5–10 min. before suckling	During suckling	2 hrs. after suckling†
Mean	2.23	1.19	6.85
Standard Deviation	2.936	0.876	9.879
Standard Error of Mean	1.199	0.358	4.418
Fiducial Limits	0–5.31	0.27–2.11	0–19.13

Standard error of the difference between the means 2.23 and 1.19 = 1.25.
Difference between these means = 0.83 × standard error.
∴ The two means are not significantly different ($0.5 > P > 0.1$).

Standard error of the difference between the means 1.19 and 6.85 = 4.43.
Difference between these means = 1.28 × standard error.
∴ The two means are not significantly different ($0.5 > P > 0.1$).

Standard error of the difference between the means 2.23 and 6.85 = 4.58.
Difference between these means = 1.01 × standard error.
∴ The two means are not significantly different ($0.5 > P > 0.1$).

the method of Bisset and Walker (7). Thioglycollic acid was adjusted to pH 7.5 with $NaHCO_3$, added immediately to part of some of the extracts to make a final concentration of 0.01 *M* at pH 7.5, and the mixtures left to stand for thirty minutes. The O.A. of the extracts that was destroyed (8) was considered as oxytocin.

In some extracts after thioglycollate treatment there was residual oxytocic activity, some or all of which could have been due to potassium or to 5-hydroxytryptamine (HT). When these were present as interfering substances, they were removed from the extract by dialysis for thirty minutes through a cellophane membrane against running distilled water, and when necessary Dibenamine hydrochloride (5 μg./ml.) was added to the 10-ml. organ bath.

RESULTS

Reference to Table 1 shows that acid-alcohol extracts of blood from lactating (primiparous) women possess oxytocic activity and that this activity is due entirely to a substance which is oxytocic in its action but which, since it is stable in thioglycollate, is not considered to be oxytocin. The table also clearly shows that there are no significant differences in O.A. (and therefore O.S.) between the blood samples collected immediately prior to suckling, during suckling, or two hours after suckling, when the infants were fed every four hours.

Table 2 demonstrates that in nonlactating mothers also the O.A. of the blood is due to O.S. and, further, that the blood levels of O.S. in the two types of mothers are not significantly different from one another. Four-point assays were carried out on the pooled extracts of the blood collected from the 6 nonlactating mothers. The close parallelism between oxytocin and O.S. in the extracts of blood is revealed in Figure 1.

TABLE 2. OXYTOCIC ACTIVITY OF ACID-ALCOHOL EXTRACT OF BLOOD OF NONLACTATING MOTHERS*—INCLUDING STATISTICAL RESULTS

Number	Total oxytocic activity	Oxytocin†	% Oxytocin / Total activity	% 2nd Oxytocic substance / Total activity
	mu/ml. blood			
1	0.93	0	0	100
2	0.80	0	0	100
3	0.98	0	0	100
4	0.93	0	0	100
5	1.42	0	0	100
6	1.05	0	0	100
Mean	1.02	0	0	100

* Mothers with bottle-fed babies between 3 and 7 months old.

† The extracts were dialyzed and the oxytocin values are uncorrected for loss through the dialysis membrane, where the mean loss of oxytocin is 50 per cent.

Total oxytocic activity of blood in mu/ml. (oxytocin)	
Mean	1.02
Standard Deviation	0.213
Standard Error of the Mean	0.087
Fiducial Limits (P=0.05)	0.796–1.244
(P=0.01)	0.669–1.371

Standard error of the difference between the means 2.23 and 1.02=1.201.
Difference between these means=1.01×standard error.
∴ The two means are not significantly different (0.5>P>0.1).

Standard error of the difference between the means 1.19 and 1.02=0.368.
Difference between these means=0.46×standard error.
∴ The two means are not significantly different (P>0.5).

Standard error of the difference between means 6.85 and 1.02=4.418.
Difference between these means=1.32×standard error.
∴ The two means are not significantly different (0.5>P>0.1).

DISCUSSION

It has been frequently stated that oxytocin is released during suckling and that this hormone facilitates the expression of milk from the mammary gland. In some species such as cows and goats this may be partly true; in these animals subsequently a conditioned (psychic) release of oxytocin develops as a result of the manipulations of the udder during the initial milkings. This conditioned release of oxytocin is possibly associated with auditory, visual or olfactory stimuli and is related to the well-ingrained habit of being milked at a specific time each day (5). The results reported here suggest that oxytocin played no part in release of milk in the primiparous women involved in these studies. The total O.A. of the blood did not rise during suckling; and this activity was due to the presence of O.S.

O.S. was not considered to be any of the known uterine stimulants such as acetyl-choline, 5-hydroxytryptamine or potassium; and the uteri used were insensitive to adrenaline and histamine. The levels of this substance in the blood during the three stages at which blood samples were collected from lactating mothers did not significantly differ from one another. It is most unlikely, therefore, that there is a reflex release of O.S., either initiated by the act of suckling or by reflexes operating through cortical association areas. This investigation gives no indication of the possible origin or role of this oxytocic substance in these women. Robertson and Hawker (9) extracted a substance with similar properties from the hypothalamus of cats (and kittens), cows and rats. It was not found in other parts of the brain, cervical cord or whole pituitary gland. The blood concentrations of O.S. in women with babies 4–7 months old who were being artificially fed because of failure of the mothers to lactate did not differ significantly from the concentrations in lactating mothers.

Acknowledgment

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THE REVERSIBILITY OF OSTEOPOROSIS IN CUSHING'S DISEASE: CASE REPORT

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ABSTRACT

The reversible nature of osteoporosis is generally accepted, but it is not often possible to demonstrate striking roentgenographic evidence of healing. A case of advanced Cushing's disease in an 11-year-old girl is presented. Osteoporosis with multiple fractures was a major cause of disability. Bilateral subtotal adrenalectomy was performed in two stages; x-ray treatment of the pituitary was given shortly after the first operation. Four years later there was remarkable x-ray evidence of the reversibility of the osteoporosis.

THE reversible nature of osteoporosis, when etiologic factors are corrected, is generally accepted (1). However, seldom is one able to demonstrate striking roentgen evidence of healing.

CASE REPORT

The patient, a Nebraska farm girl of northern European extraction, was well (Fig. 1) until the age of 11 years, at which time a huge appetite developed and she gained weight rapidly. This was followed by increasing hirsutism, acne and weakness. No known illness, or physical or emotional trauma was associated with the onset of symptoms.

FIG. 1. Patient at the age of ten years, about one year prior to the onset of her illness.

FIG. 2. Patient at the height of her illness demonstrating the classic features of advanced Cushing's syndrome. Note the extreme muscle wasting in the lower extremities.

FIG. 3. Osteoporosis with compression fractures and "codfish" deformities of the lumbar spine.

FIG. 4. Patient, aged 18 years, four years after bilateral (two-stage) adrenalectomy. The striae persisted but were pale. Posture was normal and there was no back pain.

About eight months after the onset, while her hair was being brushed vigorously, she was suddenly seized by severe pain in the middle of her back. This later became more severe and generalized, so that eventually the entire spine was painful and motion was severely limited.

She had had three very scanty spontaneous menstrual periods. The first occurred at the age of 11½ years (*after* the onset of the illness).

She was first seen in the Shelton Clinic about one year after the beginning of her symptoms. Weakness was so extreme that she was confined to a wheelchair.

Figure 2 demonstrates the classic appearance of advanced Cushing's syndrome in this girl. Physical examination revealed extreme obesity, a plethoric moon-shaped face, and acne. There was moderate hirsutism over the trunk, and especially on the back and face. The skin was thin and bruised, with many livid basophilic striations over the abdomen, buttocks and legs. Blood pressure was 144/110 mm. Hg, and the pulse was 112. There were scattered fine rales in the chest, and she was mildly dyspneic when at rest in bed. The bones were tender, especially over the vertebral spines, but also over the sternum, ribs and tibial regions. Muscle wasting was extreme.

Table 1 summarizes the representative laboratory data. Upon x-ray examination, the skull was normal except for precocious calcification of the pineal body. There was advanced osteoporosis of the bones of the spine and the pelvis, with fractures (Fig. 3).

After appropriate preoperative preparation the abdomen was explored at St. John's Hospital, Santa Monica, California, by Dr. William Longmire. No ovarian or adrenal tumors were found. The left adrenal, which weighed 8.8 grams, was removed. The postoperative course was stormy, and the patient nearly died. Following surgery, in early 1952, she was given x-ray therapy[1] to the pituitary in a total dosage of 2650 r in 12 treatments (KVP 220, MA 15, distance 50 cm., thickness 16.5 cm.). There was temporary symptomatic and chemical evidence of improvement, but before very long she had reverted to her former condition. About one year later the right adrenal, weighing 10 grams, was removed by Dr. Mark Rabwin through a flank incision through the bed of the eleventh rib.

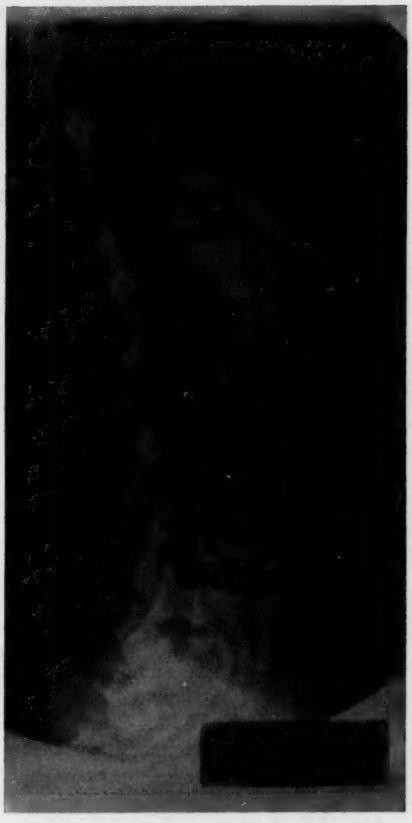

FIG. 5. Lumbar spine, four years after total adrenalectomy. Note remineralization and more normal contours of the vertebral bodies.

[1] By Dr. John J. Jares, radiologist.

TABLE 1. SUMMARY OF LABORATORY DATA

	Preoperative	Postoperative
Urinalysis	pH 6.0 (repeatedly acid) Albumin 3+ Sugar 2+ Numerous granular casts	pH 5.0 Albumin 0 Sugar 0 Rare casts
Circulating eosinophils	66 per cu. mm.	260 per cu. mm.
Glucose tolerance (100 Gm. of glucose in 1 oral dose)	Blood sugar levels: fasting 133 mg. per 100 ml. 1 hour, 182 mg. 2½ hours, 222 mg. 4 hours, 167 mg.	Blood sugar levels: fasting, 97 mg. per 100 ml. 1 hour, 122 mg. 2½ hours, 118 mg. 4 hours, 108 mg.
Serum sodium	148 mEq./L.	144 mEq./L.
Serum chloride	96 mEq./L.	123 mEq./L.
Serum potassium	2.1 mEq./L.	3.8 mEq./L.
Urinary 17-ketosteroids	18.2 mg./24 hrs.	1.7 mg./24 hrs. Patient then given 25 mg. of ACTH i.v. over 8 hours on 3 successive days: 17-KS 2.5 mg., 1st day 2.9 mg., 2nd day 1.9 mg., 3rd day
Urinary 11-oxysteroids	1.8 mg./24 hrs.	

After the second operation she was maintained on cortisone acetate (12.5 mg. twice daily), Percorten trimethylacetate (37.5 mg. intramuscularly once monthly) and salt by mouth. Postoperatively, menses were regular, and she grew 5.2 inches, became virtually asymptomatic, and was able to attain almost perfect school attendance. The acne and hirsutism disappeared and the striae became pale and inconspicuous.

Figure 4 shows the patient's appearance at the age of 18 years—four years after total adrenalectomy.

Bone disease was an outstanding feature of this patient's disease Figure 3 demonstrates the extreme radiolucidity with "codfish" deformities and compression fractures typical of osteoporosis. Figure 5 shows the remarkable healing of the bone disease four years later. Back pain had disappeared and there was no other orthopedic disability. No specific orthopedic measures were employed, such as traction, immobilization or braces. Elimination of the etiologic factor, excessive corticosteroid secretion, apparently accounted for the healing. The fact that the patient still had some growth potential may have contributed to the remarkable changes.

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THYROID FUNCTION IN SUBACUTE THYROIDITIS

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ABSTRACT

The status of thyroid function during the course of subacute thyroiditis has been assessed in 56 cases, 21 of which received no specific therapy to modify the natural course of the disease.

In 11 very severe cases the initial extensive inflammatory necrosis presumably led to flooding of the body with thyroid hormone, thus producing symptoms of hyperthyroidism with a high level of serum protein-bound iodine (PBI), an increased basal metabolic rate and a profound depression of thyroidal I^{131} uptake to virtually zero. Following the acute phase the patient passed through a euthyroid interval into a phase characterized by symptoms of hypothyroidism with a low level of serum PBI. During the later part of this phase the ability of the gland to concentrate iodine began to increase. During the recovery phase, the thyroidal I^{131} uptake rose to levels above normal in a rebound phenomenon, whereas the serum PBI gradually rose to levels that did not exceed the normal range.

In 15 moderately severe cases, the acute phase was similar in character but usually less severe. The thyroid glands of patients in this group were not sufficiently damaged to cause a hypothyroid phase. After the serum PBI level fell to a normal range, the ability of the gland to take up radioiodine also returned to normal, without the rebound phenomenon. In the 30 milder cases, there was neither a hyperthyroid nor a hypothyroid phase, but the thyroidal uptake of I^{131} was usually depressed for several weeks. In all cases there appeared to be complete recovery with no permanent sequelae.

Roentgen-ray or cortisone therapy modified the symptoms greatly. However, the phase of temporary hypothyroidism was not averted by therapy in very severe cases, and it is difficult to be certain (because of individual variations) whether or not the *total* duration of the thyroiditis was shortened.

THE purpose of this paper is to describe and discuss the disturbance in thyroid function which has been noted in patients with subacute thyroiditis. Subacute thyroiditis may be defined as an acute, subacute or chronic self-limiting inflammation of the thyroid gland, of unknown etiology. It is characterized by fever, malaise and a firm, painful and very tender thyroidal enlargement. It runs a variable, often prolonged course

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but almost always subsides without functional impairment of the gland. It is an entity separate and distinct from Hashimoto's or Riedel's struma and is more common than both of these conditions together. Although the clinical and pathologic features of this disease have been described in numerous publications, there are relatively few reports which deal in detail with the altered thyroid physiology (1–9). Investigators who have dealt with this aspect of the disease usually discuss the biochemical findings in the acute phase of the illness and do not describe the changing pattern of thyroid function throughout the entire clinical course.

Thus, the elevation of the serum protein-bound iodine level associated with a marked depression of the thyroidal uptake of radioactive iodine is a well known feature of the acute phase. The elevated level of protein-bound iodine in severe cases is frequently associated with a clinical state simulating hyperthyroidism. However, it has not been generally appreciated that, following the subsidence of the acute phase, several patients go through a stage of transient hypothyroidism associated with a low concentration of serum protein-bound iodine. McConahey and Keating (2) reported that myxedema occurred in 6 of their 21 patients in a late stage of the disease, but they did not stress its transient nature. It was to study the changing pattern of thyroid physiology, both in the natural course of the disease and in cases in which the course was modified by therapy that this investigation was undertaken.

SELECTION OF CASES AND METHODS

The clinical and laboratory findings in 56 cases of subacute thyroiditis were studied. All but 5 cases were drawn from the hospital records of the Toronto General Hospital and the private files of one of the authors (M.W.J.). The 5 exceptions were obtained from the private records of our colleagues.

Thirty-five of the 56 patients were treated with roentgen-ray, cortisone or surgical therapy; 21 were followed through the unmodified natural course of the illness. Twenty-nine patients returned during the early months of 1956 for follow-up examinations, and the present clinical status of all but 6 of the remainder was ascertained by letter. The clinical course of these patients is described elsewhere (10). The cases were divided into three groups, depending upon the nature and severity of the disease: 1) very severe; 2) moderately severe; 3) mild.

Using the method of Barker *et al.* (11), the serum protein-bound iodine concentration was determined in 44 of the 56 patients, often serially. In our hands the normal range has been found to be between 3.5 and 6.5 μg. per 100 ml. (12). The thyroidal uptake of radioactive iodine was measured in 40 patients. A tracer dose of 5 to 15 μc. of I^{131} was injected intravenously and the uptake was measured twenty-four hours later by means of a scintillometer placed 15 cm. from the estimated center of the thyroid gland. Whenever warranted, further localization of activity was made by means of a localizing cone. The basal metabolic rate was determined on 19 patients but serial records were not obtained.

RESULTS

Very severe cases

During the clinical course of the 11 extremely severe cases, 4 phases could be discerned both by clinical observation and laboratory procedures (Figs. 1 and 2). In the early acute stage of the disease there was (a) a *thyrotoxic phase* which subsequently subsided through a (b) *euthyroid phase* which might be so short-lived as to pass unnoticed into a (c) *hypothyroid phase;* later there was a (d) *recovery phase* as thyroid function returned to normal. Eleven of our 56 cases could be classified as very severe.

(a) *Thyrotoxic phase:* These patients frequently presented with a sudden fulminating onset, severe pain in the region of the thyroid gland with radiation to the ears, high fever and a pronounced systemic reaction with malaise, fatigue and weight loss. Examination revealed a moderately enlarged, extremely firm and very tender thyroid gland or part of the

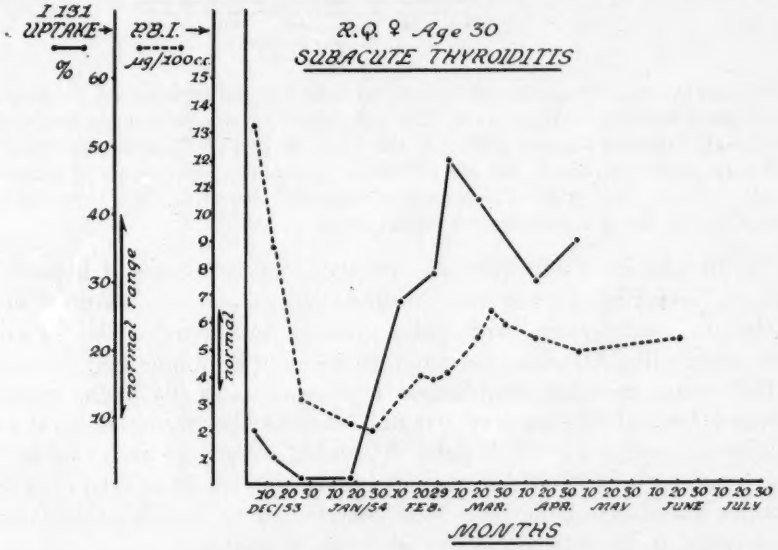

FIG. 1. Thyroidal I^{131} uptake and serum protein-bound iodine (PBI) levels in an untreated very severe case of subacute thyroiditis.

The first determinations were made six weeks after onset. Note the initially high PBI values and the extremely low I^{131} uptakes. The PBI values dropped rapidly from hyperthyroid levels, through the normal range, to subnormal levels. The stage of transient hypothyroidism lasted about two months. With recovery, the I^{131} uptake rose to high (above normal) levels as the PBI rose to normal levels; the uptake of I^{131} then fell to within the normal range.

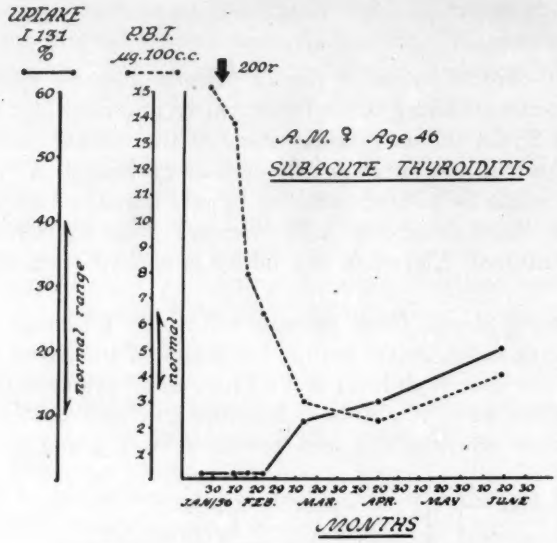

FIG. 2. Thyroidal I^{131} uptake and serum PBI levels in a very severe case of subacute thyroiditis treated by roentgen rays. The first determinations were made two weeks after onset. Roentgen therapy (200 r) to the whole thyroid gland resulted in relief of local pain and tenderness. It did not prevent the subsequent appearance of transient hypothyroidism, from which the patient is presently recovering. The thyroidal I^{131} uptake has not (as yet) risen beyond normal levels.

gland. In addition there were usually signs and symptoms of hyperthyroidism, including nervousness, restlessness, insomnia, sweating, heat intolerance, tachycardia, wide pulse pressure and tremor. The patients were acutely ill. This phase persisted for weeks or even months.

The serum protein-bound iodine level was unusually high, ranging between 7.9 and 15.1 µg. per 100 ml., whereas the thyroidal uptake of radioactive iodine was profoundly depressed, usually to zero (Table 1). This combination of findings was noted for several weeks or even for a few months. Basal metabolic rates were determined on 5 such patients and were found to lie between +20 and +42 per cent.

The thyrotoxic phase persisted until the serum protein-bound iodine level fell to within the euthyroid range. The local manifestations as well as the malaise, lassitude and fever often began to abate as this stage ended, but this was not always true. X-radiation frequently appeared to arrest the acute stage of the disease but did not necessarily prevent the subsequent hypothyroid phase.

(b) *Euthyroid phase:* This was actually an intervening zone through which the patient passed fairly quickly from the hyperthyroid to the

TABLE 1. SERUM PROTEIN-BOUND IODINE LEVEL AND THYROIDAL UPTAKE OF RADIOACTIVE IODINE IN VERY SEVERE CASES OF SUBACUTE THYROIDITIS

Patient	Sex	Age	Hyperthyroid phase				Euthyroid phase		Hypothyroid phase			Recovery		
			Time after onset 1st values obtained	Highest PBI (μg./100 ml.)	I^{131} uptake (%)	Duration (wks.)	I^{131} uptake (%)	Approx. duration (wks.)	I^{131} uptake (%)	Lowest PBI (μg./100 ml.)	Duration (wks.)	Highest I^{131} uptake (%)	Eventual PBI (μg./100 ml.)	Eventual I^{131} uptake (%)
A. *Untreated*														
R.Q.	F	30	6 wks.	13.3	8	8	0	1	0–27	2.0	2	48	4.5	35
G.J.	F	46	9 wks.	8.9	0	11	0	2	0–12	0.9	7	67	6.0	36
A.L.	F	37	3 wks.	14.2	0	7	0	1½?		1.9	4	44	5.1	18
F.R.	F	39	4 wks.	14.6	0	10	—	2	1	2.4	4	40	5.4	21
L.S.	F	56	—†			10 approx.	—	?	0	1.4	Desicc. thyroid started; not discont.			
D.E.	F	32	—†			9 approx.	—	?	6	1.2	Desicc. thyroid started; discont'd 18 mos. later		4.3	18
B. *Treated with Roentgen Therapy:*														
N.B.	F	42	8 wks.	7.9	1	11	1	1?	15	2.2	3	45	4.3	19
R.G.	F	56	4 wks.	7.2	—	5	—	?	1	0.6	Desicc. thyroid started; discont'd 1 yr. later		3.6	10
A.M.	F	46	2 wks.	15.1	0	4	0	2	0–12	2.2	6	Under observation		
I.C.	F	27	2 wks.	11.9	0	3½	—	?	5	2.0	Desicc. thyroid started; not discont'd			
L.M.	F	35	3 wks.	13.0	6	5	Given Lipiodal elsewhere, thus vitiating further data				8 approx.	2 approx.		

* In all but 1 case, first protein-bound iodine values were the highest obtained.
† The patient was not directly observed in this phase, but there was a good history of it.

hypothyroid phase. Often, but not invariably, the local pain and tenderness had subsided and the patient felt well. Even though no longer tender, the thyroid gland was still enlarged and hard. Some untreated patients continued to have local pain and tenderness associated with malaise and lassitude, during this interval but the manifestations suggesting hyperthyroidism had disappeared.

The level of serum protein-bound iodine fell, sometimes abruptly, to within the euthyroid range. The thyroidal I^{131} uptake remained very low during this phase, which usually lasted only one to three weeks.

Several patients who had been severely ill proceeded from this euthyroid phase to complete recovery without passing through a phase of hypothyroidism, the destruction of thyroid tissue presumably being less complete than in the others. These cases were classified in the *moderately severe* group.

(c) *Hypothyroid phase:* The euthyroid phase passed gradually into the hypothyroid phase, which was characterized by increasing sluggishness, drowsiness, irritability, dry hair and skin, bags under the eyes, intolerance to cold, a low husky voice and a slow pulse. Slowed cerebration and pseudomyotonia were not noted. In all but 1 patient, the local symptoms in the neck had already subsided but the thyroid gland remained hard and enlarged, although not tender, for weeks or months.

Of the 11 patients who passed through this hypothyroid stage, 5 had been given x-ray therapy during the acute phase, but 6 had received no specific therapy. The radiation therapy had caused a rapid cessation of the acute phase in 4 of the 5 patients. During the hypothyroid phase, 4 patients were given desiccated thyroid and this treatment was maintained for at least a year. In 2 of these patients administration of the drug was discontinued at the end of one year, and subsequently thyroid function was found to be normal; the other 2 patients could not be persuaded to discontinue this medication. The remaining 7 patients went through this period of temporary hypothyroidism without thyroid therapy despite the moderate discomfort involved; the period lasted from two to seven months with eventual, though gradual, recovery.

The concentration of serum protein-bound iodine fell to hypothyroid levels during this period—sometimes as low as 0.6 μg per 100 ml. (Table 1). Although the thyroidal uptake of radioactive iodine was often low at the onset of the hypothyroid phase, it subsequently began to rise, presumably as thyroid recovery commenced. There was an inevitable time lag before the serum protein-bound iodine level began to follow the rising radioactive iodine uptake (Figs. 1 and 2).

(d) *Recovery phase:* Following the end of the hypothyroid phase, the patients progressed to full recovery. By this time the thyroid gland usually

had returned to its normal size, shape and consistency. However, in 1 patient there was recurrent thyroid swelling and tenderness for seven months following recovery from the hypothyroid period; the local symptoms then subsided permanently.

The thyroidal I^{131} uptake continued to rise during the recovery phase, often rebounding to levels that were higher than normal; in 1 instance an uptake of 67 per cent was reached (G.J., Table 1). The values then subsided to within the euthyroid range, where they remained. The serum protein-bound iodine level followed the rising I^{131} uptake until it reached the euthyroid zone, where it tended to level off; it did not rise above normal as did the thyroidal I^{131} uptake.

Moderately severe cases

In general, these patients were less severely ill than the group described previously. In a few cases the early manifestations were very severe but, as in all the other patients in this group, the disease passed through only two phases—a thyrotoxic period and a recovery period. The destruction of the gland was not sufficiently complete to cause even temporary myxedema. Fifteen patients were in this category.

The serum protein-bound iodine levels and thyroidal uptakes of radioactive iodine in a fairly characteristic case are charted in Figure 3.

(a) *Acute phase:* This requires no further clinical description, as it was of the same nature but usually less pronounced than in the group of very severe cases.

In this stage the serum protein-bound iodine level was elevated, although usually less than in the very severe group. Basal metabolic rates were determined in 5 patients during this period and they varied between +10 and +20 per cent. The thyroidal I^{131} uptakes were markedly depressed, usually to zero, just as in Group I. In some instances, although only one lobe seemed clinically involved, there was no uptake of I^{131} in either lobe.

(b) *Recovery phase:* Recovery followed subsidence of the acute phase, which sometimes lasted several weeks. As the acute phase ended, the systemic manifestations cleared and the local pain and tenderness subsided, leaving a firm or hard, enlarged but non-tender thyroid gland. The patients began to feel fairly well at this stage. Several days or weeks later the gland softened and finally returned to normal size, shape and consistency.

The clinical changes were mirrored in the laboratory findings. The concentration of serum protein-bound iodine fell to the euthyroid level, where it remained. It did not drop to a subnormal level and hence there was no hypothyroid phase. The thyroidal I^{131} uptake rose from the initially low

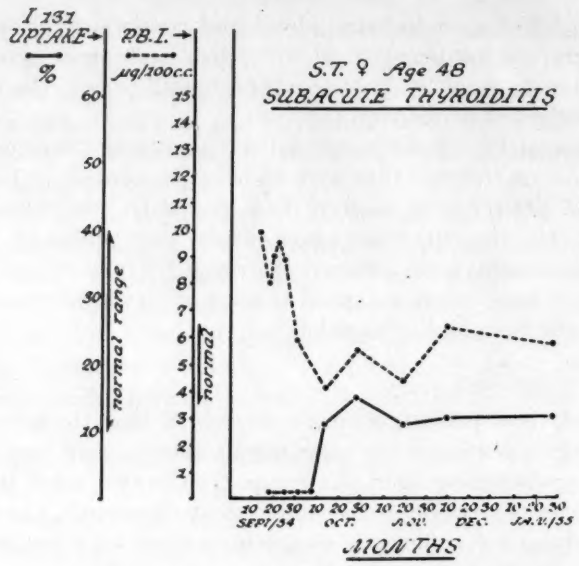

FIG. 3. Thyroidal I^{131} uptake and serum PBI levels in a moderately severe case of subacute thyroiditis in which there was fairly rapid recovery without specific therapy. The first determinations were made four weeks after onset. The hyperthyroid phase subsided to a euthyroid state and the extremely low radioiodine uptake soon rose to normal, without intervening hypothyroidism. (Aside from shortened acute phases, similar curves were obtained in patients treated by roentgen therapy.)

level into the euthyroid range; it remained there and did not exhibit the rebound phenomenon seen in the recovery phase in some patients who went through a hypothyroid phase.

Mild cases

(a) *With systemic symptoms:* There were 21 patients in this group. Although the thyroid was painful and tender, sometimes severely so, the systemic signs and symptoms were less striking than in the other groups. Malaise and fatigue were often prominent, but fever and manifestations suggesting a hyperthyroid state were either minimal or absent. The mild systemic symptoms usually cleared with subsidence of the local pain and tenderness, either spontaneously or with therapy, but in some cases malaise and lassitude persisted for some weeks after the local signs had subsided.

Laboratory evidence of disturbed thyroid function was less than that noted in the moderately severe cases. The basal metabolic rate, measured in 6 patients, varied between −6 and +10 per cent. The serum protein-bound iodine values were within the normal range in the acute phase and

did not vary significantly throughout the course of the disease. The thyroidal uptake of radioactive iodine was extremely low in most instances, although in 4 cases it was normal or only slightly low. In the patients with initially depressed uptakes, the values gradually returned to normal after a few weeks and remained there, manifesting only normal fluctuations (Fig. 4).

(b) *Without systemic symptoms:* There were 9 patients in this group. The thyroidal pain and tenderness were moderate, slight or (in 4 cases) absent, although a firm, enlarged gland was palpable in all instances. Systemic symptoms were minimal or absent. The diagnosis is more difficult when both local and systemic symptoms are very mild, the temporary enlargement of the thyroid being the chief or only manifestation. In 5 of these patients there was an enlarged, firm or hard, tender gland suggestive of subacute thyroiditis; and the subsequent course, with recovery in a few months, was in keeping with this diagnosis. The other 4 patients had an enlarged thyroid with neither pain nor tenderness. Two had been operated on and the diagnosis was made upon histologic examination of the tissues. Recent painless enlargement of the gland had suggested the diagnosis in

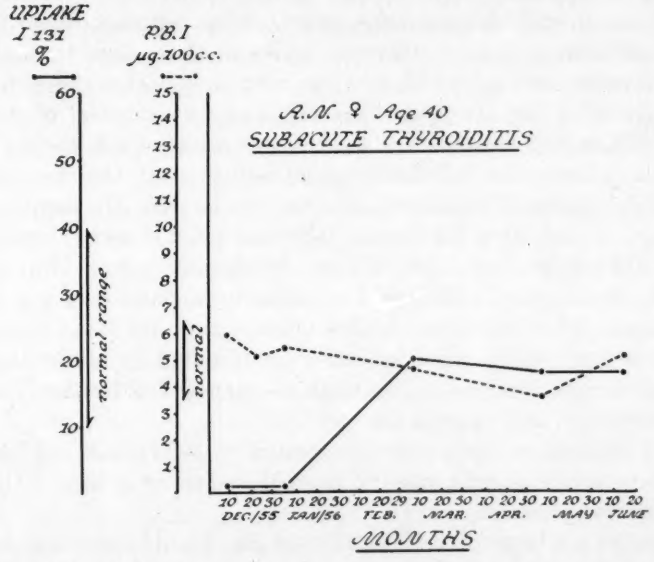

FIG. 4. Thyroidal I^{131} uptake and serum PBI levels in a patient with mild subacute thyroiditis who received no specific therapy. The first determinations were made four weeks after onset. The serum PBI values remained normal throughout. The initially depressed I^{131} uptake soon returned to normal. (Similar curves were also obtained in mild cases treated by roentgen rays.)

the other 2; the finding of a low thyroidal I^{131} uptake seemed to confirm it; and the swelling subsequently disappeared in a characteristic manner.

The serum protein-bound iodine level was measured in 7 of these patients and was normal throughout the entire clinical course. Only one basal metabolic rate was determined and it was normal (+2 per cent). The thyroidal I^{131} uptake was determined in 7 cases; it was depressed in 4 but subsequently returned to normal. In 3 patients who had painful, tender glands, the I^{131} uptake was normal, with normal fluctuations throughout the course of the illness.

Effect of treatment on thyroid function

Only two forms of therapy appeared to have a specific effect in subacute thyroiditis, namely, steroid therapy and x-ray therapy.

Twenty-four of the 56 patients received deep x-ray therapy to the thyroid gland; the routine total dosage of between 200 and 400 r was given in four fractional doses. The whole gland was irradiated (with 1 exception) even if only one lobe seemed to be involved. In 17 of the patients so treated, the local pain and tenderness began to improve within a few days following therapy and cleared entirely within the next fortnight. The acute systemic manifestations subsided similarly. Seven patients did not respond well to one course of x-ray therapy, and 4 of these were treated again. Although radiation therapy seemed to arrest and shorten the acute phase of the illness, it *did not prevent the subsequent development of temporary hypothyroidism* in 5 patients nor shorten this phase of the disease. Only 1 of these 5 patients had failed to respond well to x-ray therapy; the other 4 gained prompt relief from local pain and tenderness. The serum protein-bound iodine level often fell quickly following x-ray therapy; however, the thyroid did not recover for a further considerable period, during which there was depletion of colloid and consequent production of a temporary hypothyroid phase. It is possible that in some cases the x-ray therapy was given at a time which coincided with the termination of the thyrotoxic phase. Eleven patients passed through the hypothyroid phase; 6 of these had not received any specific therapy.

Only 3 patients in this series were treated with cortisone. All had either moderately severe or mild cases of thyroiditis and none passed through a a hypothyroid phase.

In 9 patients a large part of the thyroid gland had been removed by the surgeons before referral. The symptoms in 8 had subsided quickly after the operation; in the ninth, the disease involved the other side after a hemithyroidectomy.

Late sequelae. One patient was followed for sixteen years but the majority were followed for only a few years. There were no proven cases of perma-

nent myxedema; however, 2 of the patients who were given desiccated thyroid therapy during the hypothyroid phase refused to discontinue this medication. (It is likely that these patients do not need the desiccated thyroid.) In a patient who had characteristic subacute thyroiditis in 1944 followed by recovery, an enlarged thyroid nodule was found in 1951. Histologic examination, showed pronounced squamous metaplasia of the nodule and a pathologic diagnosis of adenoacanthoma was made. Although no further treatment was given, the patient has since been well. In none of our patients did hyperthyroidism follow the thyroiditis.

DISCUSSION

Correlation of pathologic and physiologic changes

It is interesting to correlate, as far as one can, the pathologic changes in subacute thyroiditis with the clinical and physiologic variations. We had only a limited amount of pathologic material for study and in most cases we had no opportunity to study the patients before surgical measures were undertaken. No needle biopsies were attempted. It is possible, however, to correlate the observations in general.

In any one gland many phases of the pathologic process are often found, although one phase or another may predominate. The acute phase is characterized by congestion with marked edema, the inflammatory changes progressing quickly to necrosis of the follicular epithelium. Whether or not there is a phase of glandular stimulation accompanying the initial congestion is a matter for speculation. Soon the body is flooded with thyroid hormone and, at this stage, necrosis of the epithelium is a prominent feature. These damaged or destroyed cells cannot concentrate iodine or continue to produce hormone. The phagocytic response, involving infiltration of histiocytes and giant-cell formation, soon begins and the lesion acquires a granulomatous appearance. The patient passes from an acute thyrotoxic phase through a brief euthyroid stage to the phase of hypothyroidism if the damage has been severe and extremely extensive.

Regeneration of follicles can be seen early in the severe granulomatous phase, and later it is a prominent feature during recovery. The rate at which these pathologic changes progress is extremely variable, as is that of the clinical and physiologic changes. Eventual recovery seems to be virtually complete, although some fibrosis may persist in the interstices between normal-appearing follicles.

In cases in which only one lobe appears to be involved, but there is depression of the radioactive iodine uptake over the entire thyroid, it may be that the process affects the whole gland to a lesser degree, interfering with its function. However, it is even more likely that, in view of the high hormone content of the blood resulting from the severe damage to one part

of the gland, the degree of pituitary stimulation becomes inadequate to maintain the function of the uninvolved parts of the thyroid. Such a conception would explain the cases in which there were acute symptoms in the early stage and later recovery without a definite phase of hypothyroidism.

It would be expected that, as the damaged cells recover and increase in size under stimulation by the anterior pituitary, there should be a strong rebound in the ability of the severely damaged thyroid to take up iodine; this period of unusually high uptake would be terminated as the serum protein-bound iodine rises to euthyroid levels which are maintained by normally functioning tissue. One would expect also that when the damage to the gland is very diffuse but not extremely severe there would be insufficient necrosis to cause a flooding of the body with thyroid hormone yet sufficient inflammatory reaction to interfere with the ability of the cells to concentrate administered iodine. Perhaps the damaged gland is still able to maintain a normal concentration of thyroid hormone in the blood by virtue of its stored colloid which, as pointed out by Fraser and Harrison (7), may be sufficient for a two months' supply. In a still milder form of focal or even fairly diffuse inflammation, the ability of the cells to concentrate the iodine might be maintained.

Comparison of subacute thyroiditis and radiation thyroiditis

It is of interest to compare the course of the lesion of severe subacute thyroiditis with that induced by radiation (with radioactive iodine in cases of angina in which the dose given was insufficient to destroy the thyroid) without complete destruction of the gland. In both instances there is fairly diffuse involvement of the thyroid, that following radiation being more uniform in distribution. In both, necrosis of the follicular cells leads to a flooding of the tissues with thyroid hormone, so that the serum protein-bound iodine level rises and symptoms of hyperthyroidism appear. This phase tends to be more severe in subacute thyroiditis. In both, the patient may pass through a short euthyroid phase to a stage of temporary hypothyroidism. In both, during the recovery phase, the ability of the thyroid gland to take up radioactive iodine increases and may rise to levels above normal in a rebound phenomenon. In both, the rise of the serum protein-bound iodine level occurs later and seldom exceeds euthyroid values. In both, there is considerable individual variation in the severity and duration of the hyperthyroid and hypothyroid phases and in the rate of recovery. In severe subacute thyroiditis the damage to the gland may be so great that its function tends to be completely lost, but apparently the destruction is not permanent no matter how severe the disease (13, 14). In either case, the remaining viable cells undergo hyperplasia under the influence of pituitary stimulation, so that complete recovery occurs. In subacute thy-

roiditis cellular destruction is never total, whereas following radiation in sufficiently large doses the gland may be totally destroyed.

Sequelae

In our cases there were no definite serious sequelae. Six years after the attack of thyroiditis, 1 patient was found to have a nodule of squamous metaplasia. Histologic examination showed adeno-acanthoma. Following local excision of the nodule, there was no extension of the lesion over the subsequent five-year period. No cases of either permanent myxedema or persistent hyperthyroidism have been observed. Recently, however, Sheets (15) reported a case of hyperthyroidism occurring several months following an attack of subacute thyroiditis; and Perloff (16) , in a review of his cases of subacute thyroiditis, reported that in 5 patients hyperthyroidism followed upon the thyroiditis.

Effect of therapy on the natural course of the disease

In the accompanying tables and illustrations it may be seen that the sequence of changes was the same whether the disease was allowed to pursue its natural course or was modified by radiation therapy. The treatment relieved the symptoms and seemed to shorten the acute phase but it did not prevent the development of hypothyroidism, even when the inflammatory process was greatly reduced. In milder cases, radiation usually afforded quick relief and the patient became free of symptoms; the swelling and hardness of the gland did not subside, however, for weeks or months. The data are not adequate for the purpose of discerning whether or not the *total* course of this disorder, *i.e.*, the notably variable time necessary for the gland to return to its normal size and consistency, is shortened by this therapy.

Our data are also inadequate for judging whether cortisone therapy, which may relieve the signs and symptoms even more quickly than radiation, actually shortens the total duration of the disease.

Acknowledgments

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Addendum

Subsequent to the preparation of this paper, we have observed 2 patients with subacute thyroiditis whose clinical courses were considered noteworthy. In one, hyperthyroidism developed following recovery from the hypothyroid phase of subacute thy-

roiditis. In the other, following recovery from the hypothyroid phase of the disease, no rebound phenomenon was observed. The radioactive iodine uptakes did not reach levels above normal, but gradually rose to within the normal range, as did the concentration of serum protein-bound iodine.

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SOME ASPECTS OF THYROIDAL IODINE METABOLISM IN A CASE OF IODINE-INDUCED HYPOTHYROIDISM

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ABSTRACT

A case of iodine-induced hypothyroidism is described. Quantitative information is presented concerning the iodine metabolism of the thyroid, with and without iodide blocking of hormonal synthesis. The following physiologic abnormalities were evident from the results of I^{131} tracer tests: greatly increased thyroidal iodide space, increased rate of transfer of iodide into the thyroid from plasma, and inhibition of organic binding of iodide. Hyperactivity of the iodide-accumulating function of the thyroid in the presence of high serum iodide levels offers no support for the theory that iodide inhibits the secretion or the action of TSH. One year after terminating all exogenous iodine and thyroid medication in this patient there was no laboratory or clinical evidence of any residual thyroid abnormality.

THE development of myxedema following the administration of large doses of iodide has been observed in hyperthyroid subjects (1–3), in euthyroid subjects with simple colloid goiter (4, 5), and in euthyroid subjects with no history of thyroid abnormality (6–10). Although information is not available concerning the mechanism by which hypofunction of the thyroid is produced in the first two groups, results of radioiodine studies suggest that the hypothyroidism observed in some subjects of the last group is due to failure of synthesis of hormonal iodine in the presence of elevated plasma and thyroidal iodide levels (7, 8, 10).

Excessive exogenous iodide has been thought to affect thyroid function in several ways: by blocking hormonal synthesis, at least temporarily (11–14); by reducing hormonal secretion from the gland (15); by inhibiting the action of thyrotropin (TSH) at the thyroidal (15, 16) or at the pituitary (17) level; or by inactivation of TSH (18). Investigation of the dynamics of iodine metabolism in the relatively rare cases of iodine-induced hypothyroidism affords an opportunity to elucidate some of the mechanisms involved in the thyroidal inhibition.

In the following case report of iodine-induced hypothyroidism, quantitative information is presented concerning the metabolism of iodine by the

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thyroid gland, with and without an iodide-induced block of hormonal synthesis.

CASE REPORT

A.P., a Negress from the British West Indies, had been under medical care for bronchial asthma since 1940. She was first seen at Lenox Hill Hospital in 1945 at the age of 49 and was treated for chronic bilateral glaucoma and bronchial asthma. There was no family history of thyroid or other endocrine disorders. The obstetrical history included a stillbirth and 2 normal children delivered by cesarean section. There was no history of intake of a goitrogen or of an unusual diet.

In 1945 the patient weighed 120 pounds, the thyroid gland was not palpable, and the blood count and urine were normal. In 1947 she weighed 142 pounds, an electrocardiogram (ECG) was normal and a chest roentgenogram showed a normal-sized heart. In 1949 her weight was 159 pounds, and an ECG showed nonspecific changes in the ST and T waves. In 1951 she weighed 175 pounds and the ECG records were similar. The patient appeared hypothyroid. She was obese, had a grating voice, dry skin and pasty complexion, and moved sluggishly. The thyroid gland was diffusely enlarged and was estimated to be three times normal size.

The patient had received treatment with bronchodilators beginning in 1940. Although it was unknown to us at the onset of study, iodide-containing medication had been prescribed at least as early as May 1949. Iodide was taken in varying amounts until it was discontinued at the time we first became aware of this history and its significance. The patient subsequently received iodide only for purposes of study.

METHODS

A.P. received intravenous tracer doses of I^{131} ranging from 28 μc. to 100 μc., between February 1953 and November 1955. Following administration of each of these doses, radioactivity over the neck region was assayed continuously for a half to one and a half hours and at intervals thereafter. Urine specimens were collected at appropriate intervals. Thyroidal radioactivity (corrected for extrathyroidal radioactivity) and the renal clearance of radioiodide were determined according to methods described previously (19). A dose of 1 to 2 grams of potassium thiocyanate (KSCN) was administered orally at various times following some of the tracer doses in order to discharge I^{131} not bound in organic form. Methods for assay of radioactivity in the administered doses and in specimens of urine, plasma and red cells have been described (20). Concentrations of total iodine and of protein-bound iodine in serum were determined[1] according to the method of Barker (21). The difference between these two values was considered to be the level of serum iodide.

The ability of the thyroid to concentrate iodide (trapping) can be measured directly only when organic binding of iodide is completely blocked. In the presence of such a block, thyroidal radioactivity following intravenous administration of radioiodide increases with time, until a peak is reached; subsequently it declines at the same rate as the plasma radioiodide concentration. In this study the extent of trapping was determined from the ratio of the total thyroidal radioactivity to the plasma concentration of radioactivity (thyroidal iodide space) after the peak uptake had been reached. The thyroidal iodide content was calculated as the product of the thyroidal iodide space and

[1] These determinations were made in the PBI Laboratory of the Sloan-Kettering Institute, New York.

the plasma iodide^{127} concentration. Observations were made on the effect on thyroidal iodide space (T_s) of a two-month course of Lugol's solution (10 drops a day) and of varying serum iodide^{127} levels resulting from injection of potassium iodide in a medication for asthma. The influence of cortisone (100 mg. per day by mouth, for ten days or longer) on the thyroidal iodide concentration gradient was also determined. The volume of plasma containing the amount of iodide transferred to the thyroid trap per unit of time (iodide influx volume flow) was calculated from an analysis of thyroidal uptake and plasma radioactivity curves, as described previously (22). This value was compared with the plasma iodide clearance rate when binding occurred. From the relationship between these two values, an estimate was made of the rate of organic binding by the unblocked gland (22).

Six months after the patient was first seen in the Isotope Laboratory, a biopsy specimen of the thyroid gland was obtained.[2]

RESULTS

A summary of the results of radioisotope studies and of the pertinent laboratory and clinical information is given in Table 1. Characteristic of Test A through D was a rapid early uptake of radioiodine, reaching peak values of 38 to 47 per cent of the administered dose two to three hours after intravenous injection of I^{131}. The absence of significant binding of radioiodide is demonstrated by the parallel decrease of plasma and thyroidal radioactivity curves beginning several hours after administration (Fig. 1A) and the dischargeability of thyroidal radioactivity by KSCN after one day (Fig. 1B). During this time, unknown to us, the patient was taking iodide in varying amounts and for two months prior to Test C she had received additional iodide as Lugol's solution (10 drops per day). Assuming the patient was in equilibrium with respect to inorganic iodide intake and excretion at the end of this period, the serum iodide level at the time of Test C was calculated to be greater than 300 micrograms per 100 ml.[3] The thyroidal iodide space was essentially constant and determined to be 23 liters in Test B, 19 liters in Test C, and 22 liters in Test D when the serum iodide level was chemically determined as 20 micrograms per 100 ml. Thus the thyroidal iodide space did not change significantly with an approximately fifteen-fold change in serum iodide level.

From the data obtained in Test D (Fig. 2), the mean rate constant for transfer of iodide from thyroid to plasma was determined to be 9.7×10^{-3}

[2] Microscopic examination showed papillary proliferation with congested capillaries as well as some distended follicles containing unevenly stained colloid. The appearance of this section was consistent with a diagnosis of thyroid hyperplasia.

[3] Renal clearance of iodide = 20 liters/day. Additional iodide as Lugol's solution ~60 mg./day. Estimated serum iodide level from known iodide intake ~3 mg./liter. Since the patient was receiving, in addition, varying and unknown amounts of iodide in asthma medication, it is probable that the serum iodide level was considerably in excess of 300 micrograms/100 ml.

TABLE 1. LABORATORY AND CLINICAL DATA IN PATIENT A.P.

Date	Test	Thyroidal I^{131} uptake (%)				Th. Cl.,* ml./min.	Ts† liters	R. Cl.,‡ ml./min.	Serum		Medications	Wt., lbs.	Serum cholesterol, mg./100 ml.	BMR, %	Thyroid gland, est. size	Remarks
		½ hr	1 day	2 days	3 days				Iodide^{127}, μg./100 ml.	PBI^{127}, μg./100 ml.						
1953 Feb. 2	A	41	28		8			25				175	308	−22	3× norm.	Hypothyroid (see clinical history)
Feb. 10	B	35	23	12	7		23	27			Lugol's sol., 30 drops/day, Feb 14–24, 10 drops/day Feb. 24–28					
Apr. 28	C	27	33				19	15	300 (calc)		KSCN, 1.5 Gm. (oral), at 24 hrs.					
Aug. 3	D	24	25	13	8		22	35	20	1.8						Clinically unchanged—biopsy of thyroid
Aug. 18	E	45	49	43	41	228		16				169	355	−28		
Oct. 26	F	51	81	81	73	355		18	2	4.7	KSCN, 1.5 Gm. (oral), at 72 hrs.	170	300	−26	3× norm.	Clinically unchanged
Nov. 27	G	27			12		13	41	138	3.2		169		−9		No change in size of heart on X-ray exam.
Dec. 11	H	26			3 (4 days)		14	36	239	3.2	Desicc. thyroid, 90 mg./day, Dec. 15–June 15			+6		
1954 Feb. 11	I	None detectable							56	7.6		168			2× norm.	Slightly less hypothyroid—thyroid gland smaller, softer
June 15											Asthma capsules without KI begun June 15			+2		Considerably improved—less hypothyroid
July 29	J	20				111		29			KSCN, 1.0 Gm. (oral) at 3 hrs.	161				
1955 Jan. 27	K	10				51		20		4.8	KSCN, 1.5 Gm. (oral) at 1½ hrs. Lugol's sol. 10 drops/day, Jan. 27–Nov. 29	149			1.5× norm.	No symptoms. Patient gradually becoming euthyroid
Sept. 20	L	5						27			KSCN, 1.0 Gm. (oral) at 2½ hrs.	145				
Nov. 15	M	6					1.9	31			Cortisone, 100 mg./day, Nov. 20–29	153				
Nov. 29	N	3					0.6	37			KSCN, 1.5 Gm. (oral) at 2½ hrs.					
1956 Dec. 12	O	6				30		28	0	6.6		158	303		barely palpable	Roentgenogram of heart showed slightly smaller size than in 1953. ECG partly reverted toward normal. Patient continued euthyroid.

* Thyroid clearance.
† Thyroid space.
‡ Renal clearance.

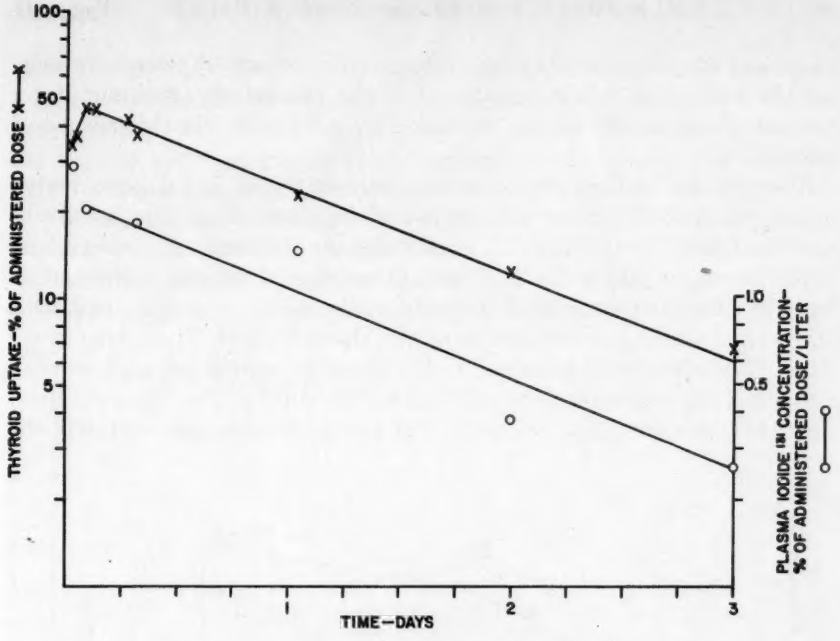

FIG. 1A

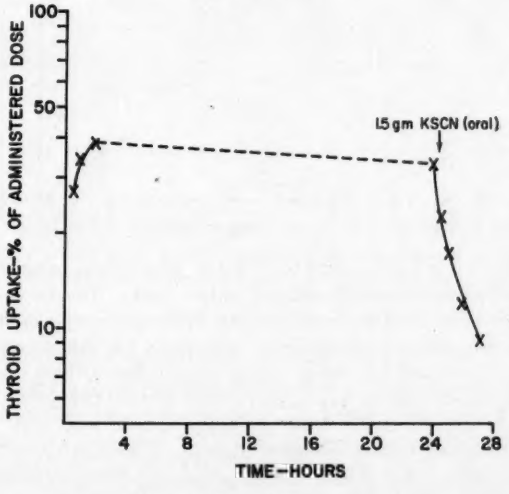

FIG. 1B

FIG. 1. A. Thyroidal uptake of radioactivity reached its peak in about two hours and subsequently declined at the same rate as the plasma iodide[131] concentration (Test B, Table 1). B. Thyroidal radioactivity was virtually all discharged by KSCN administered at the end of the first 24 hours. At this time the renal iodide clearance was only about 15 ml./min., resulting in a slow rate of removal of iodide[131] from the body (Test C, Table 1).

* Thyroid clearance.
† Renal clearance.

min^{-1} and the mean iodide influx volume flow (volume of plasma containing the amount of iodide transferred to the thyroid trap per unit time) was calculated as 216 ml. per minute. Figure 2 also shows the method of calculation.

Two weeks after Test D, presumably because the subject did not receive iodide medication during this period of hospitalization, the results of another tracer study (Test E) were strikingly different. At twenty-four hours the thyroidal uptake of I^{131} was 49 per cent of the administered dose and the subsequent course of thyroidal radioactivity was consistent with the normal binding of radioiodine by the thyroid gland. Thus, even at six days, 33 per cent still remained in the gland as contrasted with a mean value of 7 per cent at three days in the earlier studies. The value obtained for thyroidal radioiodide clearance (228 ml. per minute) was virtually the

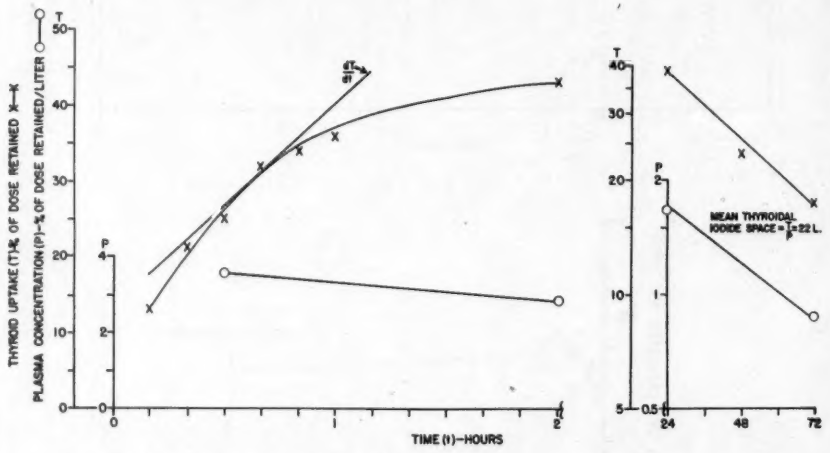

FIG. 2. (Test D, Table 1.) From 24 to 72 hours there was a parallel decrease in thyroidal uptake and plasma concentration of radioactivity. The thyroidal iodide space (T_s) was calculated to be 22 liters, from the ratio of thyroidal uptake (T) to plasma concentration (P) of radioactivity. Methods for calculating the rate transfer of iodide from thyroid to plasma (λ_{TP}) and the iodide influx volume flow (which under conditions of total block of binding is equal to $\lambda_{TP}T_s$) have been derived previously (22).

$$\lambda_{TP} = \frac{dT/dt}{PT_s - T}$$

where dT/dt, the slope of the thyroid uptake curve, P, the plasma concentrations of radioactivity and T, the thyroid uptake are evaluated at the same time (in the figure, at the 40-minute point). The mean value given in the text ($\lambda_{TP} = 9.7 \times 10^{-3}$ min^{-1}) was obtained from evaluating λ_{TP} at several times between 30 and 60 minutes. The mean iodide influx volume (216 ml./min.) was calculated from the product of the mean value of λ_{TP} and the mean thyroidal iodide space (T_s).

same as that which had been obtained in the previous study for the mean iodide influx volume flow (216 ml. per minute). This agreement indicates that binding must have occurred extremely rapidly, as it usually does in the thyroid gland of euthyroid and hyperthyroid subjects (22). Plasma PBI^{131} at three days was 0.019 per cent of the administered dose per liter, and at six days was 0.034 per cent per liter.

When the patient returned for evaluation two months later, all medication was stopped for a two-week period. At this time her serum iodide level was 2 micrograms per 100 ml. and a tracer study (Test F, Fig. 3)

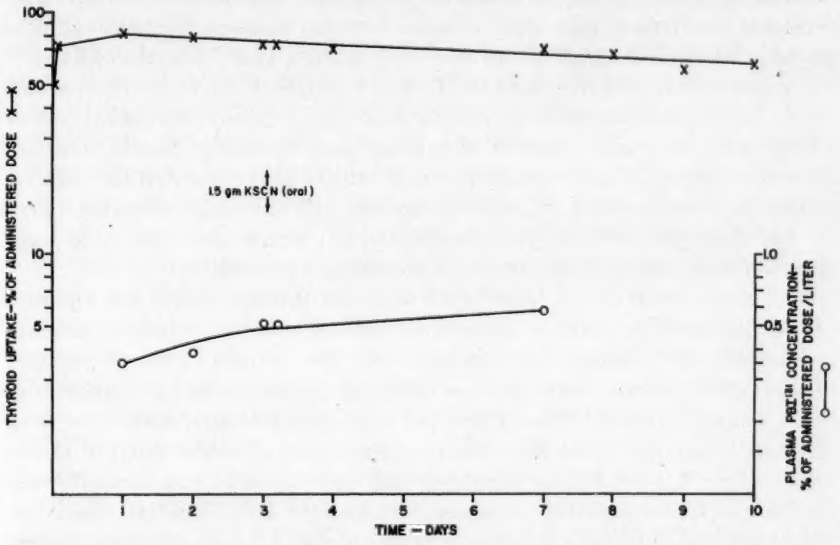

FIG. 3. (Test F, Table 1.) The prolonged retention of thyroidal radioactivity, even following administration of KSCN, indicates no block of binding of iodide. The PBI^{131} plasma concentration at 48–72 hours was near the upper limit of normal for this laboratory (20).

showed rapid binding of administered radioiodide. The thyroidal radioactivity remaining three days later was not dischargeable with KSCN. The plasma PBI^{131} level at 48–72 hours was near the upper limit of the normal range for this laboratory (20). The serum PBI concentration had increased to 4.7 micrograms per 100 ml., from a level of 1.8 micrograms per 100 ml. obtained two months earlier. Following her discharge from the hospital, the patient resumed the asthma medication, and repeated tracer studies (Test G, Test H) showed again the characteristic block of organic binding with serum iodide levels of 138 and 239 micrograms per 100 ml. respectively, and with thyroidal iodide spaces of 13 liters and 14 liters re-

spectively. The thyroidal iodide content was 18 mg. in Test G and 33 mg in Test H. The serum concentration of PBI was 3.2 micrograms per 100 ml. Although no decrease in the size of the gland could be observed by palpation, the thyroidal iodide space had decreased to about 60 per cent of the previous level.

Desiccated thyroid was started in a dosage of 90 mg. daily. After two months the thyroidal clearance and uptake of radioiodide were negligible (Test I), the serum PBI level was 7.6 micrograms per 100 ml., and the patient was clinically euthyroid. Following another four months of therapy with desiccated thyroid, the size of the gland had definitely decreased. Desiccated thyroid was discontinued but the asthma medication, prepared without KI, was continued. One month later, the thyroidal I^{131} clearance was 111 ml. per minute (Test J) and KSCN given two and a half hours following intravenous administration of the radioiodide failed to discharge any detectable amount of radioactivity from the gland. After six months without iodide or exogenous thyroid, the gland was further reduced in size to about one and a half times normal, the thyroidal clearance (Test K) had dropped to 51 ml. per minute, and two hours after the tracer dose the thyroidal radioactivity was not discharged by KSCN.

The patient was given Lugol's solution (5–10 drops daily) for a period of eight months in order to determine whether a block of iodide binding, associated with increased hyperplasia, could be induced again. At the end of this period, there was no increase in the size of the gland upon palpation, but a peak thyroidal I^{131} level of 7 per cent of the administered dose was obtained from one to two hours after intravenous administration of radioiodide (Test L), and at four hours virtually all thyroidal radioactivity was discharged following administration of 1 Gm. of KSCN. After two additional months of Lugol's solution, a peak uptake of 7 per cent at two hours and a thyroidal iodide space of 1.9 liters were observed (Test M). The thyroidal iodide space had thus remained practically constant for this two-month period. Although the thyroidal iodide space did not reach the very high levels observed initially, the values obtained were considerably in excess of normal. Cortisone (100 mg. daily by mouth) was administered for ten days and the same dose of Lugol's solution was continued. Test N revealed a decrease in thyroidal iodide space to about 0.6 liter. All medication was then discontinued.

One year later the patient was clinically euthyroid. The thyroidal iodide clearance (Test O) was within normal limits (30 ml. per minute) and after four hours the thyroidal radioactivity was not dischargeable with KSCN. The serum total and protein-bound iodide levels were the same (6.6 micrograms per 100 ml.). An aliquot of the same serum was analyzed at another

laboratory[4] for both total serum precipitable and butanol-extractable I^{127}. The values for both were identical (6.5 micrograms per 100 ml.).

DISCUSSION

Although hypothyroidism following iodine treatment was first noted almost thirty years ago (1), only occasional cases had been reported (2–4) until recently, when the general use of radioiodine tests for the study of thyroid function simplified the detection and evaluation of this abnormality. A systematic study of the kinetics of iodine metabolism in this pathologic condition may provide information applicable to the study of normal thyroid physiology.

The patient described in this report had no history of thyroid dysfunction prior to administration of iodide. After four or more years of iodide therapy, she presented a clinical picture of hypothyroidism, although the thyroid itself exhibited some features of hyperactivity. The size of the gland was increased to about three times normal and the thyroidal iodide space had reached the inordinately large value of about 20 liters—some forty times normal (23). The hyperplasia was so marked that a thyroidal iodide content of more than 60 mg. was small compared to the total iodide concentrating capacity of the gland, for a greater than fifteen-fold increase in serum iodide levels from 20 micrograms per 100 ml. to greater than 300 micrograms per 100 ml. produced no significant decrease in the thyroid/plasma concentration gradient. These results would tend to discount the suggestion that iodide acts directly on the pituitary (17) or through inactivation of TSH (15–18). That component of TSH which accounts for the growth and increased concentration gradient certainly appeared augmented following prolonged iodide therapy. This report presents no information relevant to the study of Goldsmith and Eisele (15) which suggests that the effect of iodide on the slowing of the rate of release of hormone from the toxic human thyroid is probably the result of some interference with TSH at the level of the thyroid gland.

One must consider whether Patient A.P. had some pathologic defect in binding which was merely intensified during iodine administration. Some pre-existing thyroid dysfunction is suggested by those cases (4, 5) in which myxedema followed iodine administration for what was presumed to be a simple colloid goiter. The case reported by Hydovitz and Rose (24) is interesting in this respect. Although the authors suggested several possibilities which related the observed goiter and myxedema to the ingested iodine,

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[4] This analysis was made in the laboratory of Dr. Evelyn B. Man, Yale University School of Medicine, New Haven.

the inability to bind iodine was not reversible following the withdrawal of iodine medication. Furthermore since the patient became myxedematous following the withdrawal of thyroxine or triiodothyronine more than a year after cessation of iodine therapy, the relationship between iodine medication and the myxedema did not appear to be established in their subject. Two weeks after withdrawal of excessive iodide, the rapid rate of thyroidal iodide binding shown by Patient A.P., the subject of the current report, was in agreement with values previously reported for normal and hyperthyroid subjects (22). This demonstrated that the block of organic binding was completely reversible. Had there been a partial block of binding, as has been observed following radioiodine therapy for hyperthyroidism (22, 25), the method employed was of sufficient sensitivity to have detected it. In addition, one year after terminating iodine medication all serum precipitable I^{127} was butanol-extractable, suggesting the presence of only normal thyroid hormone.

Whether or not the hypothyroidism which manifested itself after at least four years of iodide administration was due principally to a block in binding, merits further consideration. During studies in which the thyroidal iodide was calculated to be greater than 60 mg., hormonal binding amounting to only 0.1 per cent of the iodide in the gland would have been sufficient to ensure a normal production of hormone. On a practical experimental basis, it is virtually impossible to detect this degree of binding. However, there is additional evidence to suggest that the block was in hormonal synthesis rather than in release of hormone from the gland. Since Patient A.P. was hypothyroid and had a serum PBI level of 1.8 micrograms per 100 ml. early in the study, it is obvious that her thyroid gland was releasing considerably less than normal amounts of hormone. Had she been producing hormone at a normal rate and releasing it at this greatly reduced rate, the difference would have been stored in the gland and would have resulted, over a four-year period, in a greatly enlarged thyroidal hormonal iodine pool. There was no evidence from the rather slow increase in the serum concentration of PBI (coupled with a high normal level of PBI^{131}) following withdrawal of iodide that the hormonal iodine pool was greatly enlarged. The picture was more consistent with that of a thyroid gland which had been depleted of hormonal iodine over a period of years. When the thyroid was no longer subjected to the depressing effect of excessive iodide, the return to normal serum PBI levels would be expected to be delayed, since even in the presence of strong stimulation by endogenous TSH an increase in the rate of hormonal release as well as an elevated uptake could not compensate rapidly for the depleted pool.

The serum iodide level which was associated with block of organic

iodine binding in Patient A.P. (between 2 and 20 micrograms per 100 ml.) was slightly lower than that reported by Wolff and Chaikoff to be associated with inhibition of organic binding by the rat thyroid (11) and approximates that reported by Stanley and Astwood for euthyroid and hyperthyroid human subjects (26).

Cortisone is known to reduce thyroidal iodide clearance and 24-hour uptake in normal subjects (27). The effect of cortisone was evaluated on the thyroid trap during a period when binding was continuously inhibited by exogenous iodide. Administration of cortisone for ten days resulted in a decrease in size of the thyroidal iodide trap to one third of the precortisone level. This is consistent with a presumed action of cortisone in depressing secretion of TSH by the pituitary.

Acknowledgments

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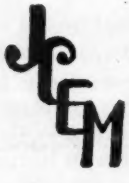

PERSISTENT THYROID CARCINOMA*

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ABSTRACT

Data regarding 17 cases of thyroid carcinoma persistent for ten to twenty-six years are reported. The ages of the patients at the time of the initial treatment varied from 8 to 56 years, and the tumors included all types of thyroid cancer. Only 1 tumor became histologically more anaplastic with persistence; all others were histologically similar at both the original and final examinations. Radical neck dissection as an initial therapeutic procedure was performed on only 3 patients. Although recurrences were limited to the neck in most instances, tumors of each histologic type showed a capacity for more distant metastases. Five of the 17 patients died as a result of persistent thyroid carcinoma.

A CHARACTERISTIC of some thyroid cancers is a slow rate of growth. Although such tumors often can be held in check or temporarily "cured" by various forms of treatment, persistence with recurrence of symptoms after five or more years is relatively common. This fact accounts for statements that ten-year (1) or even twenty-year (2) follow-up studies are necessary to evaluate thyroid carcinoma adequately.

A study of cancers persistent for long periods of time provides a unique opportunity to broaden our knowledge of the life history not only of thyroid tumors but of all cancer. Points of particular interest in such a study are: 1) the types of cancer most likely to persist locally or as metastases, 2) the frequency of the transition of one type of cancer to another, 3) the possibility of cancer becoming more malignant with persistence, and 4) the sites in which long-persistent cancers are commonly found.

MATERIAL AND DATA

The material for this study consisted of 17 cases of thyroid carcinoma persistent for ten or more years. The prerequisite for inclusion was that the malignant tumor removed at the time of the original operative procedure and also the tumor removed ten or more years later were available for histologic review. The cancers were grouped according to the classification of Warren and Meissner (3). In some of the patients recurrences were

* Presented at the Annual Meeting of the American Goiter Association, New York, N. Y., May 28–30, 1957.

TABLE 1. ORIGINAL AND FINAL TYPE OF TUMOR IN 17 CASES OF PERSISTENT THYROID CARCINOMA

Patient			Original tumor type	Years persistent	Final tumor type
No.	Age at time of first treatment (yrs.)	Sex			
1	22	F	Follicular	10	Follicular
2	54	M	Undifferentiated (giant-cell)	10	Undifferentiated (giant-cell)
3	36	F	Follicular	11	Follicular
4	53	F	Follicular	12	Follicular
5	55	F	Papillary	12	Papillary
6	55	F	Undifferentiated	12	Undifferentiated
7	55	F	Undifferentiated	13	Undifferentiated
8	56	F	Follicular	13	Undifferentiated (giant-cell)
9	53	M	Follicular	14	Follicular
10	8	F	Follicular	17	Follicular
11	25	F	Papillary	17	Papillary
12	24	F	Follicular	20	Follicular
13	36	F	Papillary	20	Papillary
14	35	F	Mixed papillary and follicular	22	Mixed papillary and follicular
15	21	F	Follicular	22	Follicular
16	31	F	Mixed papillary and follicular	24	Mixed papillary and follicular
17	11	F	Papillary	26	Papillary

frequent, but since these recurrent tumors were all similar histologically to the final one, only the last is recorded in the tables (1 and 2).[1]

As in most reported series of thyroid cancer, the majority of the patients (15 out of 17) were females. The ages of the patients at the time of the original treatment varied from 8 to 56 years; 10 patients were less than 37 years old, and the other 7 were between 53 and 56 years. The duration of persistence of the tumor varied from ten to twenty-six years; in 9 patients it was ten to fourteen years, and in 8 it was seventeen years or longer.

[1] Since this manuscript was submitted for publication, Dr. Machteld E. Sano of Newton Memorial Hospital, Newton, New Jersey, has informed the authors of the recent death of a patient in this series (Case #9). Autopsy showed tumor, histologically similar to the thyroid cancer, in the heart, lungs, kidneys, adrenals, brain, prostate, parathyroid capsules and surgical scar tissue.

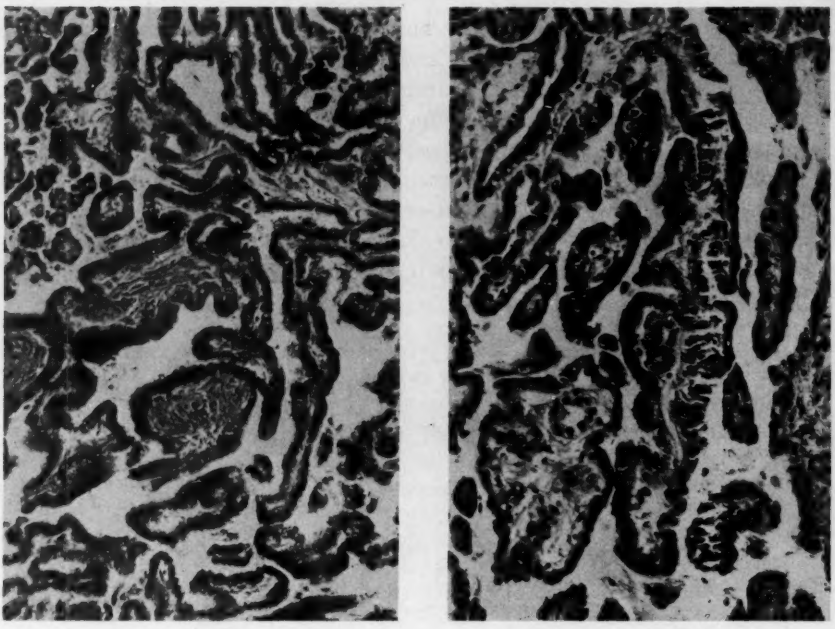
The type of the original tumor and that of the final tumor appeared histologically identical (Figs. 1, 2 and 3) with 1 exception; in Case 8 the tumor originally was a follicular carcinoma but thirteen years later it was predominantly a giant-cell carcinoma (Fig. 4). The most common type of original tumor was follicular (8 cases); there were 4 papillary carcinomas, 2 mixed papillary and follicular carcinomas, and 3 undifferentiated carcinomas (of which 1 was of the giant-cell variety).

The initial treatment (Table 2) included excision of the tumor, x-irradiation, and radical neck dissection. In 6 patients treatment consisted of local

TABLE 2. FORMS OF TREATMENT, SITES OF RECURRENCE, AND MORTALITY IN 17 CASES OF THYROID CARCINOMA

Case No.	Type of tumor	Initial treatment				Site of recurrence		Death
		Excision	Radical neck dissection	X-ray	Desicc. thyroid	Neck	Other	
1	Follicular	x			x	x		
2	Undifferentiated (giant-cell)	x		x		x		x*
3	Follicular	x				x		
4	Follicular	x				x	Brain	x
5	Papillary	x		x	x	x		
6	Undifferentiated		x	x	x		Lung	
7	Undifferentiated	x					Lung, bone, viscera	x*
8	Follicular→ Giant-cell	x		x		x	Bone, lung	x*
9	Follicular	x		x			Bone	
10	Follicular		x	x			Lung	x*
11	Papillary	x				x		
12	Follicular	x		x	x		Lung, bone, viscera	x
13	Papillary	x		x		x		
14	Mixed papillary and follicular	x				x		
15	Follicular		x	x	x	x		
16	Mixed papillary and follicular	x		x		x		
17	Papillary	x		x	x		Lung	x*

* Autopsy.



A B

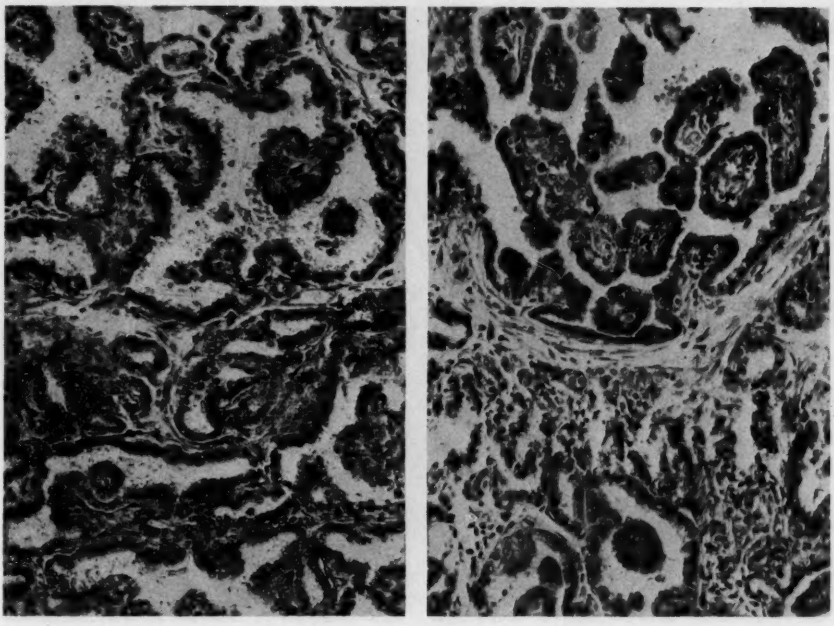
FIG. 1. Papillary carcinoma of the thyroid, persistent only in the neck for twenty years (Case #13).

The patient was a 36-year-old-woman who in 1931 complained of a goiter present for eight years. A papillary carcinoma (**A**) which showed blood-vessel invasion was excised locally; x-irradiation was given postoperatively. In 1936, x-irradiation was given again because of a recurrence in the neck. In 1951, an additional recurrent tumor in the neck (**B**) was excised. Note that the two specimens obtained twenty years apart were histologically identical. There was no clinical evidence of recurrence beyond the neck.

excision only; in 8, local excision followed by x-irradiation; and in 3, radical neck dissection plus x-irradiation. Six of the patients received desiccated thyroid after surgical therapy.

Persistent tumor was limited to the neck in 9 cases (Fig. 1); in 6 there was no recurrence in the neck. However, some of the patients with no cervical tumor at the time of the last treatment had had such tumors previously. In 2 patients there was tumor, both in the neck and in distant sites. Of the metastatic sites beyond the neck, the lung was the most frequent (Fig. 2); there were 6 cases of metastasis to the lung, 4 to bone, 1 to the brain, and 2 to more than one viscus (Fig. 3).

According to the most recent information, 10 of the 17 patients were alive,[1] 7 had died, and autopsies had been performed on 5. Among the 7



A B

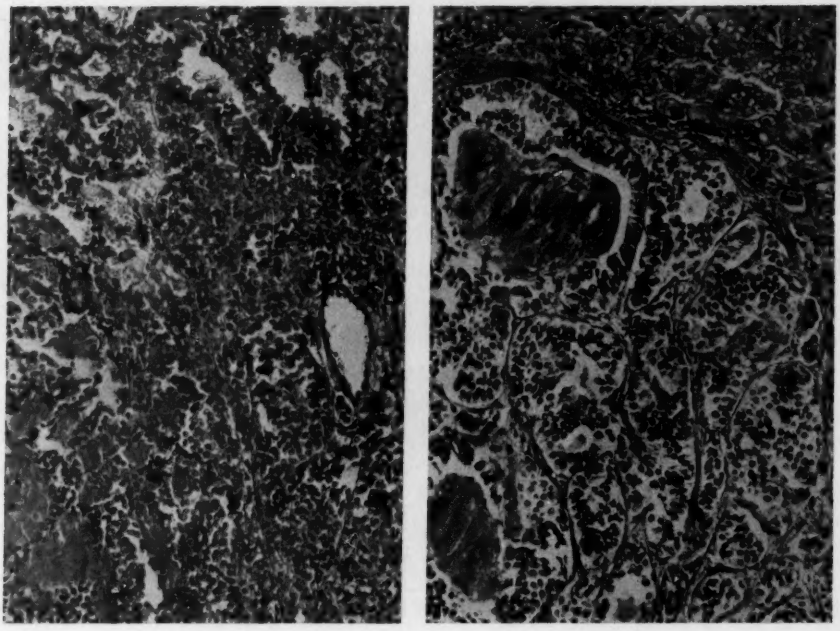
FIG. 2. Papillary carcinoma of the thyroid persistent for twenty-six years, metastatic to the lung (Case #17).

An 11-year-old girl in 1930 had had a nodule in the neck for one and a half years. Local excision of a papillary carcinoma (**A**) which showed blood vessel invasion was followed by x-irradiation. Several recurrences in the neck were treated by surgery or x-irradiation and the patient received desiccated thyroid. In 1956, twenty-six years after the initial surgical procedure, papillary carcinoma (**B**) was removed from the right lung and hilar lymph nodes. Both the original tumor and the lung metastasis showed foci of epidermidization as well as "psammoma" bodies. The patient died a few months after the last procedure, from a malignant ependymoma; no residual thyroid tumor was found at autopsy.

deaths, 5 were attributed to thyroid cancer—2 from local extension, 2 from disseminated metastases, and 1 from metastasis to brain.

DISCUSSION

It is apparent from this study that prolonged persistence of thyroid cancer cannot be predicted on the basis of the histologic type. In this series, every major type of thyroid cancer was observed, including undifferentiated carcinoma of the giant-cell type, which is usually considered to be the most malignant of all thyroid tumors (4). Papillary carcinomas (which as a group are accepted as being of low-grade malignancy) were



A B

FIG. 3. Visceral metastases of follicular thyroid carcinoma after twenty years (Case #12).

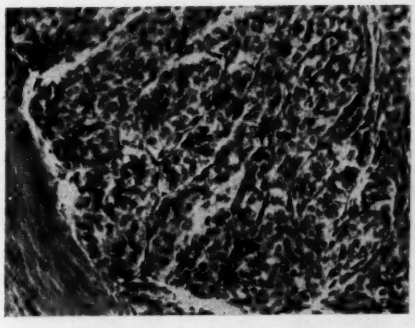
The patient was a 24 year-old woman. In 1932 a follicular carcinoma (**A**) showing foci of undifferentiated carcinoma and blood-vessel invasion was excised locally. Postoperatively, treatment consisted of x-irradiation and desiccated thyroid. In 1952, twenty years after the initial operation, multiple metastases were evident clinically, and were confirmed histologically by biopsy of the liver (**B**) and ovary. The patient died the following year, presumably from widespread metastases. No autopsy was performed.

found in only 4 of the 17 cases, although in 2 others there were papillary foci. It is noteworthy that in only 1 instance did a tumor change its type and become more anaplastic. The foregoing observations, coupled with the fact that most thyroid cancers do not have a long persistent course (5), imply that the growth behavior of any form of thyroid cancer may vary from patient to patient but tends to remain constant in the individual case.

Long persistence of thyroid cancer is not limited to persons in the younger age groups, since 7 of the 17 patients were more than 52 years of age when treatment was initiated. This is of particular interest, as 3 of the patients in the older age group had undifferentiated carcinoma.

It is remarkable that patients are able to survive so many years with

A



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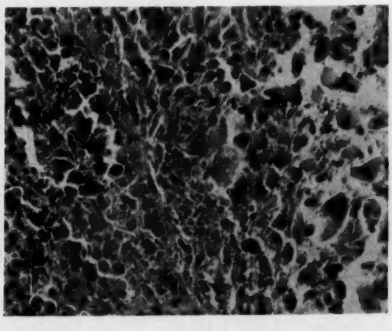

C

FIG. 4. Follicular thyroid carcinoma with ultimate change to giant-cell carcinoma after thirteen years (Case #8).

A 56-year-old woman complained in 1942 of a goiter which had been enlarging for fifteen years. A follicular carcinoma (**A**) was excised. In 1955, thirteen years after the original excision, the patient died with massive recurrent tumor in the neck and metastatic tumor in bones and viscera. Some of the persistent tumor (**B**) was similar histologically to the original, but much of it was of the giant-cell type (**C**).

persistent malignant disease, and that they are not more incapacitated. As far as can be determined, only 5 patients of the 17 died as the result of thyroid cancer. One explanation may be that most recurrences were limited to the neck—either the soft tissues, lymph nodes or residual thyroid tissue. The low-grade and localized nature of cervical recurrences suggests that continued and aggressive treatment is indicated.

The initial treatment in the majority of these patients was excision, often followed by x-irradiation, but without radical neck dissection. Only 3 of the patients underwent radical neck dissection as the original therapeutic procedure. It would seem that, as a group, these patients with persistent thyroid cancer did not have what would be considered to be either vigorous or even adequate initial surgical treatment. Whether or not more aggressive therapy would have resulted in complete cures is problematical, as it is impossible to draw conclusions from the available data.

For considerable periods of time 6 of the patients were given desiccated thyroid, presumably not as a treatment for the cancer but as treatment for the hypothyroidism that followed operation and x-irradiation. The majority of patients did not receive this medication and survived for comparable periods. Thus no conclusions regarding the effectiveness of desiccated thyroid as a therapeutic agent for thyroid carcinoma seem justified.

Acknowledgment

The authors are indebted to numerous pathologists and clinicians for assistance in collecting the data.

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THE PLASMA PROTEINS IN THYROID DISORDERS*

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ABSTRACT

Serum proteins were examined by flocculation tests and by paper electrophoresis in 246 patients. The colloidal gold test was found to be a fairly good, but not invariably reliable, criterion of Hashimoto's disease when other causes of abnormality have been excluded. The albumin/γ-globulin ratio was also found to be a useful diagnostic criterion in Hashimoto's disease. The contention could not be maintained that the primary factor in this disorder is failure of the thyroid cells. In hypothyroidism apart from Hashimoto's disease, the β-globulin fraction was increased and the γ-globulin fraction was normal. The reverse was true in Hashimoto's disease.

IN THE course of an investigation of the plasma proteins in thyroid disorders we have paid special attention to those in hypothyroidism, in Hashimoto's disease and in the condition in which lymphadenoid nodules, often associated with Hürthle cells, are found in thyroids which do not strictly fall within the definition of Hashimoto's disease generally agreed by English pathologists. By Hashimoto's disease we mean that condition in which virtually all the normal thyroid tissue has been replaced by a mixture, in varying proportions, of lymphadenoid tissue (usually arranged in nodes containing "germ centers") and Hürthle cells, accompanied by various degrees of fibrosis, with little or no storage of colloid.

METHODS

Blood was removed from 246 consecutive patients attending the thyroid clinic at New End Hospital. All specimens were examined by the colloidal gold test and those found to be abnormal were examined by paper electrophoresis. In the majority of cases other flocculation tests also were performed. Our controls consisted in part of 56 patients attending the thyroid clinic who were found not to be suffering from thyroid disorder, and in part of patients never suspected of thyroid disease who had been referred to one of us (R.G.) at the Royal Northern Hospital. Electrophoresis was performed on Whatman No. 1 filter paper in a buffer solution (pH 8.6) made up of 10.3 Gm. of sodium diethylbarbiturate and 1.84 Gm. of diethylbarbituric acid per liter. A potential of 10

* Presented at the Annual Meeting of the American Goiter Association, New York' N.Y., May 28–30, 1957.

volts per 1-cm. length of paper was applied for three and a half to four hours. The stain used was bromphenol blue, 0.1 per cent in 95 per cent ethyl alcohol saturated with mercuric chloride. For the quantitative estimation of the protein-dye complex, the strips were cut and each segment eluted with 7 ml. of 2 per cent (w/v) sodium carbonate in 50 per cent (v/v) methyl alcohol. The resulting eluate was measured with a Spekker absorptiometer using an Ilford filter No. 606. The method is essentially that of Hardwick (1). The total protein concentration was measured by the method of Phillips *et al.* (2).

RESULTS

Limitation of space forbids the presentation of results in detail. They are, however, summarized in Figures 1–5, and Tables 1 and 2.

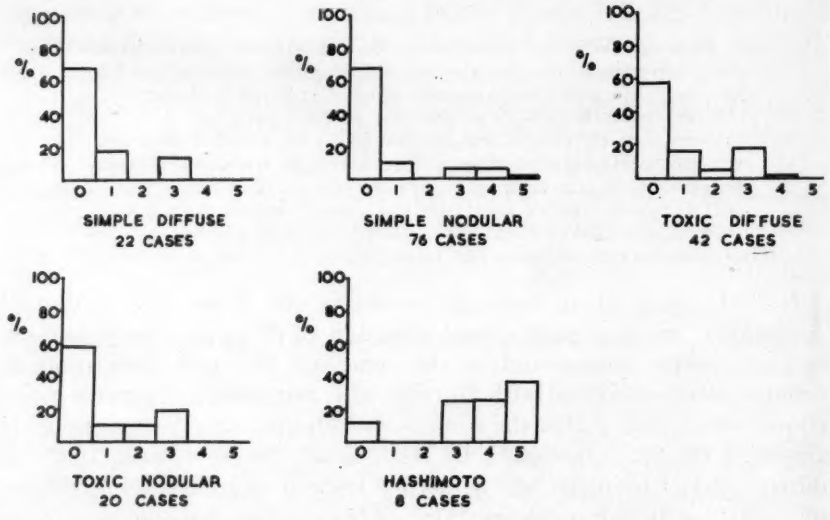

FIG. 1. Results of colloidal gold tests in thyroid disorders.

As shown in Figure 1, results of the colloidal gold test were positive in 87.5 per cent of cases of Hashimoto's disease and in 37.3 per cent of cases of other diseases of the thyroid. However, on the basis of the findings in our control study, we are disposed to accept as abnormal only values above 2 With this understanding, results of the test were positive in only 17.75 per cent of patients without Hashimoto's disease.

As may be seen in Figure 2, electrophoresis of serum from 8 cases of Hashimoto's disease demonstrated that the γ-globulin fraction was high, the β-globulin fraction normal, and the albumin fraction reduced. The results in the 8 patients with Hashimoto's disease are listed in detail in Table 1. The results in 11 patients without Hashimoto's disease but with lymphadenoid nodules in the thyroid are listed in Table 2.

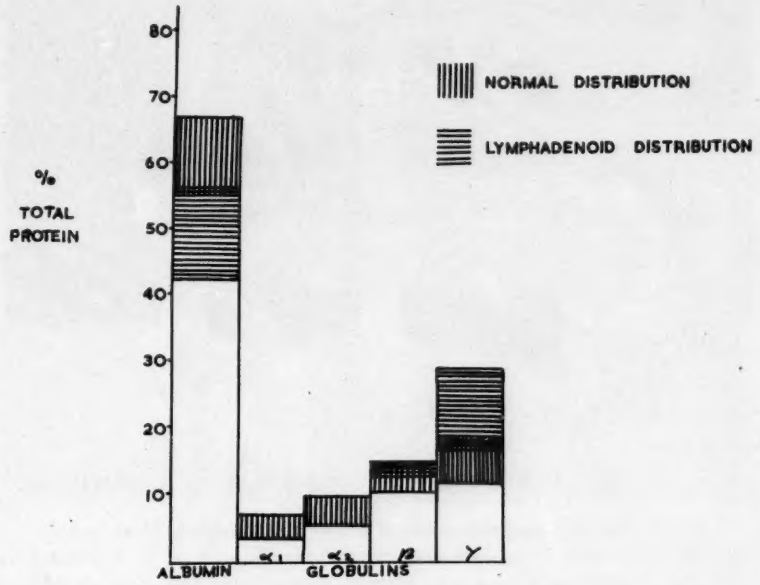

FIG. 2. Paper electrophoresis of serum proteins in 8 cases of Hashimoto's disease.

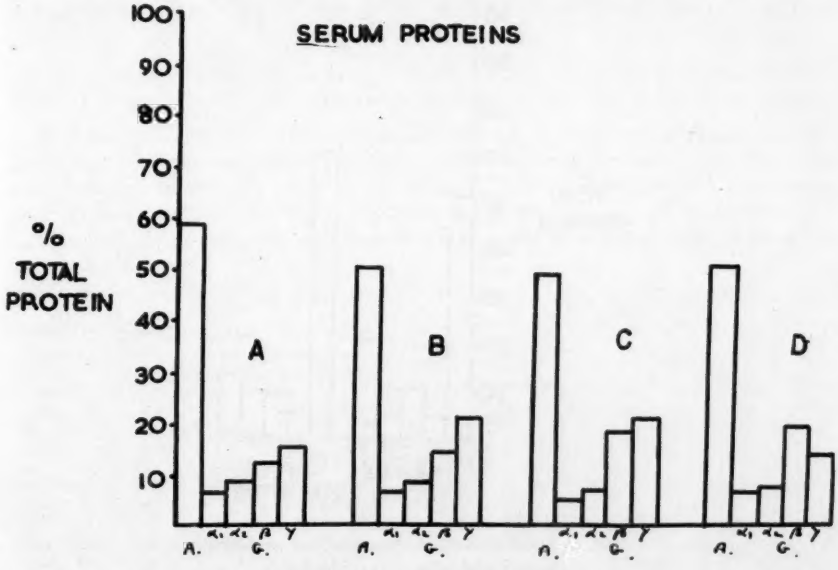

FIG. 3. The serum protein pattern in Hashimoto's disease compared with that in controls, in hepatitis and in myxedema. Group A = Normal controls. Group B = Hashimoto's disease. Group C = Hepatitis. Group D = Myxedema.

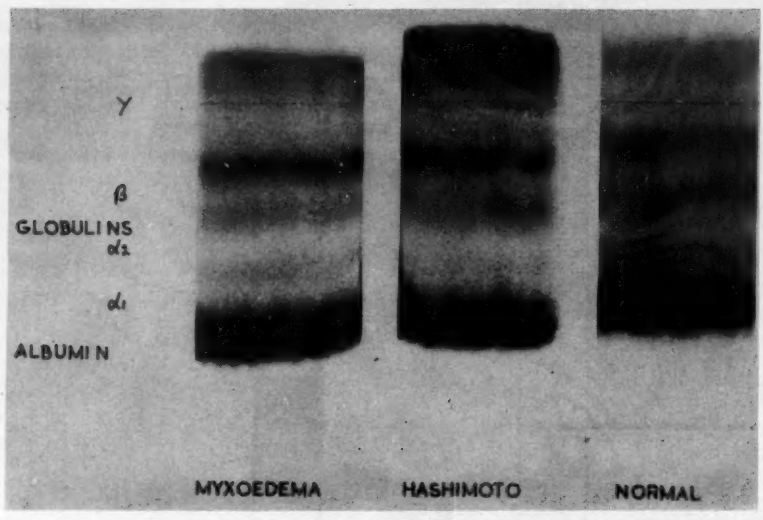

FIG. 4. Electrophoretic patterns of the serum proteins in Hashimoto's disease and in myxedema.

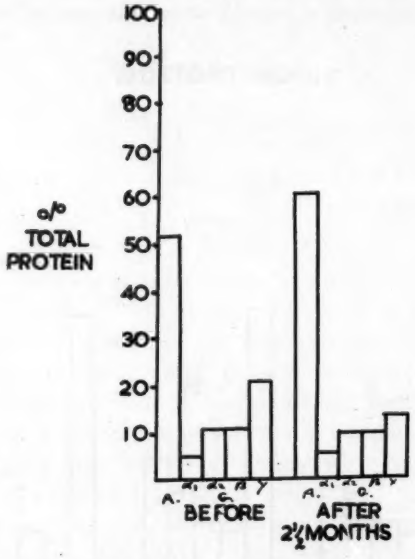

FIG. 5. Hashimoto's disease. Serum proteins before and after thyroidectomy.

TABLE 1. FINDINGS IN 8 PATIENTS WITH HASHIMOTO'S DISEASE INVESTIGATED BEFORE OPERATION

In all but 1 case the results of the colloidal gold test were abnormal. The β-globulin value lay between 11 and 16 per cent (normal = 10–14 per cent), and the γ-globulin value between 19 and 25 per cent (normal = 12–18 per cent). The thyroid index (T) was calculated from the excretion of I^{131} according to the method of Arnott and Fraser (3).

Patient		Thyroid index (T)	Serum cholesterol (mg./100 ml.)	BMR (%)	Colloidal gold	Zinc sulphate	Thymol turbidity	Total proteins (Gm. %)	Protein, fractions (%)					Ratio alb./ γ-glob.
No.	Age								Alb.	Globulin				
										α_1	α_2	β	γ	
64	58	3.6	338	−10	4	10	10	7.2	52	4	7	12	25	2.1
41a	40	2.06	236	+ 4	3	7	5	7.5	51	7	7	15	20	2.5
53a	23	1.9	300	—	4	6	5	7.7	51	7	10	13	19	2.7
75a	48	—	250	—	3	6	4	6.8	50	5	9	16	20	2.5
76a	49	—	—	−7	5	8	5	7.2	46	7	10	14	23	2.0
85a	59	—	—	—	5	—	—	—	50	6	7	11	26	1.9
S	52	—	332	—	0	—	—	6.8	66	5	8	9	12	5.5
W	50	—	422	—	5	21	18	7.9	52	5	11	11	21	2.5

In Figure 3 is shown a comparison of the serum protein pattern in Hashimoto's disease with that in controls, in hepatitis, and in myxedema. In the 5 cases of myxedema (no lymphadenoid change), the β-globulin fraction was high (15–20 per cent) and the γ-globulin fraction normal

TABLE 2. PREOPERATIVE FINDINGS IN 11 PATIENTS WHOSE GLANDS CONTAINED LYMPHADENOID NODULES BUT DID NOT CONFORM WITH OUR DEFINITION OF HASHIMOTO'S DISEASE

Results of the colloid gold test were abnormal (>2) in 4 patients (36.3 per cent compared with 17.75 per cent for all cases other than Hashimoto's disease). The β-globulin value was normal and the γ-globulin value was high in these 4 cases in which electrophoresis was performed. Thus they showed a resemblance to cases of Hashimoto's disease proper, but no statistical significance can be claimed, as the number of cases was too small.

Patient		Thyroid index (T)	Serum Cholesterol (mg./100 ml.)	Colloidal gold	Zinc sulphate	Thymol turbidity	Total proteins (Gm. %)	Protein fractions (%)					Ratio alb./ γ-glob.
No.	Age							Alb.	α_1	α_2	β	γ	
27	64	—	—	0	2.5	2							
34	49	9.6	196	3	8.0	1	6.8	54	7	5	11	23	2.3
46	48	80		4	13.0	3	7.9	44	7	8	14	28	1.6
71	23			4	9	4		50	6	9	14	21	2.4
82	39	56		0	4	1							
6A	40			0	4	2							
14a	37	8		0	8	1							
31a	53	>100		0–1									
43a	45	39		0–1	4	—							
47a	47		196	3–4	3	3		50	7	10	13	20	2.5
59a	60			0	5								

(13–17 per cent). The resemblance between the patterns in Hashimoto's disease and in hepatitis was striking.

The photographs of the paper strips (Fig. 4) show clearly the difference between the serum protein pattern in myxedema and that in Hashimoto's disease.

The results depicted in Figure 5 confirm the previous observations of Doniach and Hudson (4) that thyroidectomy may cause the electrophoretic pattern to return to normal despite the continuance of myxedema. In 1 of our patients, not treated with thyroid, the γ-globulin fraction dropped from 21 to 13 per cent and the colloidal gold curve became normal within six weeks after operation, yet the clinical signs of myxedema, the β-globulin fraction, and serum cholesterol concentration remained at their preoperative levels.

DISCUSSION

The plasma proteins in thyroid failure have been described by many authors (5–10). Almost general agreement has been reached that a characteristic change in hypothyroidism is a decrease in the albumin fraction and an increase in the β-globulin fraction; some authors report also an increase in the γ-globulin fraction. Apart from those who have confined their attention to hypothyroidism without goiter, little attempt has been made until recently to distinguish electrophoretically between different kinds of hypothyroidism. It may be significant that in their series Lamberg and Gräsbeck (10) found that the rise in globulin affected only the β fraction.

Abnormal colloidal gold curves were reported in 2 patients with Hashimoto's disease by Cooke and Wilder (11)—an observation extended by Cooke and Luxton (12, 13). They found that in 21 of 24 cases of Hashimoto's disease, the colloidal gold test yielded abnormal curves which reverted to normal after treatment with thyroid for four to twenty-six months. In 5 of 9 patients the results of the bromsulphalein test were abnormal, and in 2 out of 23 the serum bilirubin level was elevated. They found the colloidal gold test more sensitive than other flocculation tests—a finding confirmed by Skillern *et al.* (14) and by ourselves.

Skirpan *et al.* (15) examined the colloidal gold curves and the plasma cholesterol levels in 143 patients, including 19 with struma lymphomatosa. They found the modified colloidal gold test of Maclagan more sensitive than the serum cholesterol test in diagnosing thyroid failure—a finding which we have confirmed. They pointed out that the colloidal gold curve depends chiefly, but not wholly, upon the proportion of albumin to globulin; depression of albumin and elevation of globulin causes an abnormal result, as it does in hepatic disorder and in certain cases of collagen disease,

multiple myeloma, leukemia and Addison's disease (16). We have examined the electrophoretic patterns in numerous cases of these disorders, especially in hepatitis and hepatic cirrhosis, and have amply confirmed this finding.

The paper of Skillern *et al.* (14) is worthy of special notice. The authors sought to show that "struma lymphomatosa" is an expression of primary failure of the thyroid cell. Careful studies were made on 25 patients in whom a diagnosis of struma lymphomatosa had been supported by examination of a needle-biopsy specimen of the thyroid. We are not prepared to agree that this is a final test, if we are to adhere to our definition previously given. (We have often suspected Hashimoto's disease on clinical grounds and found a patchy distribution of lymphadenoid tissue, some of which might well have been picked up by a Silverman needle and led to a false diagnosis.) Moreover we are unhappy about their classification of struma lymphomatosa. Their struma lymphomatosa (Hashimoto type) is apparently what we mean by Hashimoto's disease. Their hyperplastic type seems to us to include what we would class as hyperplastic goiter associated, as it so often is, with lymphocytic infiltration and trabecular fibrosis. In our view, this bears no relationship to struma lymphomatosa. In their series, the β-globulin value was above their upper level of the normal range in 13 out of 14 Hashimoto cases but in only 1 out of 6 "hyperplastic" cases. It would be expected, if thyroxine deficiency played a major part in the changes in plasma proteins associated with Hashimoto's disease, that the β-globulin value would be increased and that of albumin decreased, at least as constantly as in cases of myxedema without a goiter.

If we may be permitted to reclassify the Cleveland cases as: 1) unequivocally hypothyroid (clinical findings supported by a low BMR or other laboratory evidence), and 2) possibly hypothyroid (laboratory evidence only), we find a high β-globulin value in 3 out of 6 of the former group and in 2 out of 5 in the latter group. In only 2 out of 8 of their patients without depressed basal metabolism, was there an elevated β-globulin value.

In the light of these findings it is difficult to share the opinion of the Cleveland workers that struma lymphomatosa should rightly be regarded as an expression of primary thyroid failure, partially or wholly compensated by an increased production of thyrotropin. We entirely agree with them that Maclagan's colloidal gold test is diagnostically the most sensitive of the tests which our team has employed (cephalin cholesterol, zinc sulphate, thymol turbidity, ammonium sulphate). However, we cannot accept the hypothesis of primary thyroxine deficiency because:

1. We are not satisfied on clinical and laboratory evidence that all of either the Cleveland patients or the Hampstead patients were in fact suffering from such a deficiency.

2. We know of no incontrovertible evidence that oxyphilia is a sign of failure of the thyroid cells.

3. We have not seen in the most severe cases of struma lymphomatosa any histologic evidence of excessive stimulation by thyrotropin. The thyroid enlargement seems to us to be due to lymphadenoid replacement and not to epithelial hyperplasia, which is usually accepted as the sign of excessive pituitary stimulation.

4. We have never seen the phenomenon mentioned by the Cleveland workers—further enlargement of the goiter after partial thyroidectomy. It is true that almost always such patients are treated with thyroxine, which would inhibit further enlargement due to thyrotropin. However, we have seen a few patients who had become myxedematous as a result of partial thyroidectomy for Hashimoto's disease, without any return of the goiter.

5. In our patients, the electrophoretic pattern of the plasma proteins often returned to normal after operation, though hypothyroidism per-

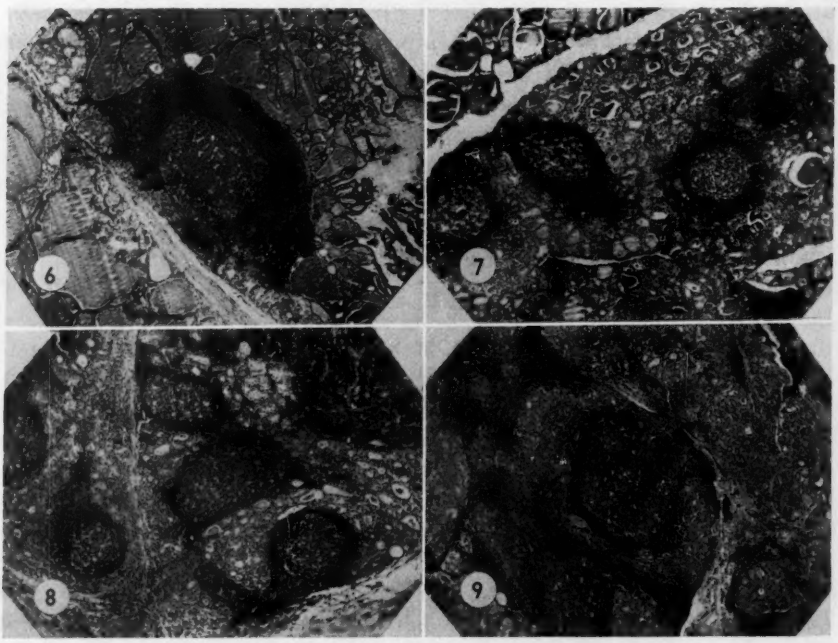

FIG. 6. A single nodule of lymphadenoid tissue in the thyroid.
FIG. 7. Scattered lymphadenoid nodules.
FIG. 8. Lymphadenoid nodules replacing thyroid tissue in large areas of the gland.
FIG. 9. Hashimoto's disease.

sisted. On the other hand, Doniach and Hudson (4) have reported and we have confirmed, that during prolonged treatment with thyroid the pattern may fail to return to normal. In 1 of our cases, treated with thyroid for four years in a dosage of 5 grains daily, values for plasma proteins did not return to normal.

6. Most important of all, the typical electrophoretic pattern in simple myxedema exhibits an elevated β-globulin fraction and a normal γ-globulin fraction, whereas in Hashimoto's disease the reverse is true. In the few cases investigated, partial lymphadenoid replacement was associated with the changes found in Hashimoto's disease.

One of us (17, 18) has previously pointed out that every histologic grade exists between a slight degree of replacement of thyroid tissue by lymphadenoid nodes and the full picture of Hashimoto's disease (Figs 6–9), and has related the degree of replacement to the chance of postoperative hypothyroidism. On such evidence alone it would be unsound to suggest that the condition involving single lymphadenoid nodes is an early or slight form of Hashimoto's disease. However, in a few cases of slight lymphadenoid replacement, results of the colloidal gold test are abnormal and the γ-globulin fraction is elevated—observations which lend support to the hypothesis.

Acknowledgments

Our thanks are due to Mr. J. E. Piercy, F.R.C.S. for permission to carry out investigations on some of his patients; to Mr. B. A. Houston, M.Sc., the Royal Northern Hospital, for the findings in flocculation tests and electrophoresis performed in non-thyroid diseases; and to Miss D. Jackson, B.Sc., for assistance in the preparation of the tables.

The authors are grateful for a grant from the Medical Research Council.

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Letters to the Editor

METABOLISM OF PROGESTERONE BY ADRENAL TISSUE FROM PATIENTS WITH CUSHING'S SYNDROME AND MAMMARY CARCINOMA*†

To the Editor:

Several steps in the biosynthesis of adrenocorticosteroids by the bovine adrenal gland have been demonstrated. Hayano *et al.* (1) and Savard *et al.* (2) demonstrated the conversion of 11-deoxycorticosteroids to glyconeogenic material. On the basis of the melting point of isolated crystals and chromatography, McGinty *et al.* (3) concluded that cortisol is the product of incubation with 11-deoxycortisol. Demonstration of the enzymic nature of the process was made by Sweat (4).

Since these original studies, 6-, 17- and 21-hydroxylations have also been demonstrated to occur in the adrenal (5–7). Recently Lombardo *et al.* (8) have reported studies on the conversion of progesterone, 17-hydroxyprogesterone and 11-deoxycortisone to cortisol in the human adrenal.

This report is a comparative study of the steroidogenic pathways in the *in vitro* metabolism of progesterone to deoxycorticosterone (DOC), 17-hydroxyprogesterone, corticosterone and cortisol by human adrenal glands from a patient with Cushing's syndrome[1] (C.S.) and a patient with mammary carcinoma[2] (M.C.).

MATERIALS AND METHODS

In each of 6 separate experiments, 2-gram portions of minced adrenal tissue were incubated with progesterone 4-C^{14} (20,000 cpm; specific activity 1.2×10^7 cpm/mg.) in a phosphate buffer at 37° C. for three and a half hours. The incubation mixtures were extracted with acetone which was filtered off and subsequently removed in a vacuum. The water residue was re-extracted with chloroform, which was removed and evaporated to dryness. The residue was redissolved in a chloroform-methanol mixture (1:1) contain-

* This work was supported by a grant from the U.S.P.H.S. (No. 34-5219).

† We are indebted to Dr. Charles Huggins and Dr. Brian Little for their cooperation in obtaining the adrenal tissues studied in these experiments.

[1] This patient was originally relieved by radiation of the pituitary. However, adrenalectomy was necessary due to recurrent symptoms. No malignant tissue was found in the adrenal glands.

[2] No abnormalities were found in the adrenal tissue of this patient.

ing 200 micrograms each of the following non-radioactive carriers: cortisol, aldosterone, corticosterone, 11-beta-hydroxyprogesterone, 17-alpha-hydroxyprogesterone and deoxycorticosterone. Chromatography was performed in the formamide systems (hexane-benzene-chloroform) of Zaffaroni (9). The steroids were identified by methods previously described (10, 11). The quantities of steroids were determined by area measurements of the chromatograms obtained on a Geiger-Mueller strip counter. Corrections for self-absorption of the C^{14} β-rays in the paper chromatograms were determined by measurements of known quantities of radioactive steroids added to samples of the same grade of paper (12).

The identification of the steroids was performed as follows: the suspected compound when chromatographed in the hexane-benzene system had the same Rf (0.62) as deoxycorticosterone. When acetylated in the presence of non-radioactive deoxycorticosterone, both acetates ran at the same rate (Rf 0.73) in the hexane-benzene system. Oxidation of both free radioactive steroid and carrier formed the etio acids which ran at corresponding rates. The specific activity of the etio acid was the same as for the preoxidized compound.

The steroid identified as 17-hydroxyprogesterone had the same Rf as deoxycorticosterone in the free form; however, when acetylated, deoxycorticosterone separates from 17-hydroxyprogesterone, since the latter does not become acetylated under the conditions employed in the tests (10). After strong oxidation 17-hydroxyprogesterone was converted to 4-androstenedione-3-17 for final identification.

The Rf of the compound identified as corticosterone corresponded, both in the free (Rf 0.75, chloroform) and acetylated form (Rf 0.66) chloroform), with the index compounds. The acetylated compound and its carrier both formed 11-dehydrocorticosterone acetate (Rf 0.73, hexane-benzene) upon oxidation with chromic oxide.

Cortisol was identified by its rate of migration in the chloroform system (RF 0.26), the rate of migration of its acetate (Rf 0.15, benzene) and the identification of the oxidation product of its acetate (cortisone acetate, Rf 0.26 benzene).

The specific activities of these latter compounds were not determined, as it was felt that 2 derivatives of the compounds were sufficient for establishing their identity (10).

RESULTS AND DISCUSSION

Neither 11-beta-hydroxyprogesterone nor aldosterone was found in the incubations. 17-Hydroxyprogesterone was not found in the products of incubation with adrenal tissue from the C.S. patient.

The total percentile conversion of progesterone incubated with adrenal tissue from the M.C. patient was 10 to 16.5 per cent (4 incubations). The respective conversions to DOC, 17-hydroxyprogesterone, corticosterone and cortisol are listed in Table 1. Corresponding data from incubations with adrenal tissue from the C.S. patient (70 and 74 per cent conversion) are also listed in Table 1.

The chromatographic patterns of the conversion products of progesterone in the 2 patients are shown in Figure 1. There was a distinct qualitative difference in the metabolic activities of the tissues from the 2 patients. Whereas a 4.5 per cent conversion of progesterone to 17-hydroxyprogesterone was noted in the incubation products with M.C. adrenal tissue, no

detectable quantities could be demonstrated in the incubation products with C.S. adrenal. The peak in the upper graph of Figure 1, which corresponds to 17-hydroxyprogesterone, proved to be deoxycorticosterone after acetylation. It may also be seen that the quantity of cortisol synthesized in the C.S. tissue was of a much greater magnitude than that synthesized in the M.C. tissue. The ratio of this steroid to corticosterone in the C.S. tissue was 1.2, and in the M.C. tissue 3.4.

These observations are consistent with those of Symington and Grant (13), who found an increased synthesis of corticosterone in the adrenals of

TABLE 1. PERCENTILE CONVERSION OF PROGESTERONE 4-C^{14} IN ADRENAL TISSUE FROM A PATIENT WITH MAMMARY CARCINOMA AND A PATIENT WITH CUSHING'S SYNDROME

	Incubation No. (2 Gm./ 2.0×10^4 cpm)	% Radioactivity calculated from radiochromatograms					Total conversion (%)
		Progesterone	DOC	17-OH-progesterone	Corticosterone	Cortisol	
I Mammary carcinoma (M.C.)	1	83.6	7.5	4.6	3.5	0.8	16.4
	2	88.2	4.4	4.3	2.3	0.8	11.8
	3	88.6	5.0	4.3	1.6	0.5	11.4
	4	90.0	3.3	4.7	1.4	0.5	10.0
II Cushing's syndrome (C.S.)	1	30.1	19.6	—*	26.8	23.5	69.9
	2	26.2	26.5	—*	25.1	22.2	73.8

* Radioactivity not detectable.

C.S. patients when deoxycorticosterone was employed as the substrate. The failure to observe 17-hydroxyprogesterone in products of incubation with C.S. adrenal tissue may have been due to either a very rapid transformation of 17-hydroxyprogesterone to cortisol, or to the existence of a pathway for cortisol synthesis in which 17-hydroxyprogesterone is not involved. The former explanation seems the more likely one, as it has been demonstrated that 17-hydroxyprogesterone can be synthesized from radioactive acetate in both ox (14) and hog adrenal preparations (15).

The percentile conversion of progesterone to cortisol in the incubated adrenal tissues from the 2 patients was markedly different. In the C.S. adrenal tissue 22.8 per cent of the steroid was converted; in the M.C. adrenal tissue only 0.7 per cent was converted. As both of these tissues were from patients with pathologic disorders, it is difficult to evaluate the data in terms of the normal adrenal. Results with adrenal tissue obtained

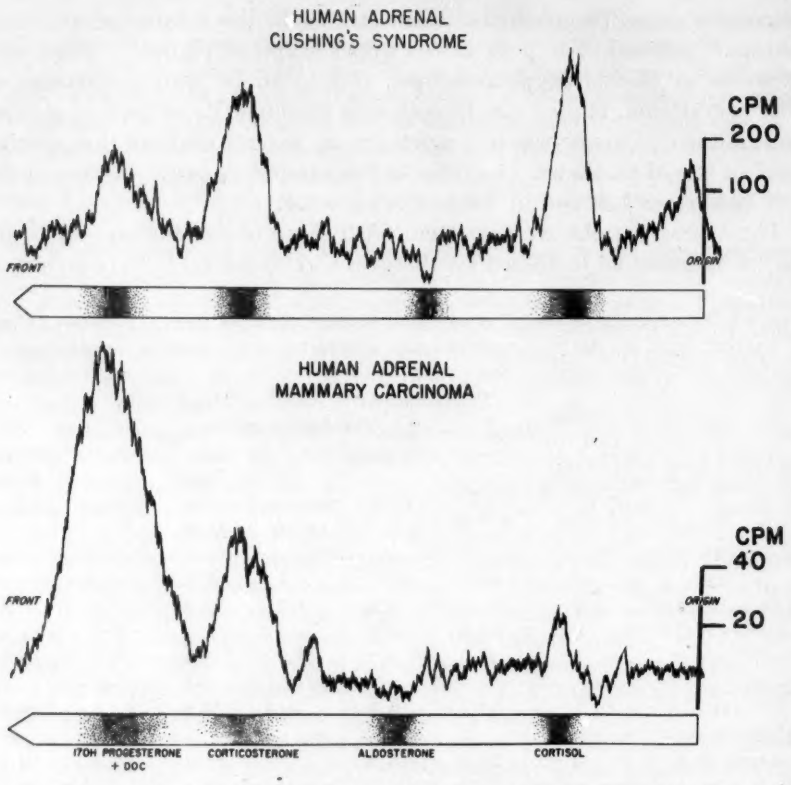

FIG. 1. Chromatographic pattern of the metabolism of progesterone-4-C^{14} by adrenal tissue from a patient with Cushing's syndrome and a patient with mammary carcinoma (chloroform-formamide system).

from the M.C. patient would probably be closest to the normal, as in this case the gross and microscopic examinations revealed no abnormal alterations in the adrenals; however, this does not exclude the possibility of metabolic aberrations not associated with structure. Moreover, the plasma steroid levels in the M.C. patient were more comparable to those in the normal subject (16).

That the *in vitro* incubation of progesterone with adrenal tissue gives rise to a greater quantity of corticosterone than cortisol, whereas human adrenal venous blood is known to contain greater quantities of cortisol (16–19), may be due to one or more of the following reasons:

1. Progesterone may not be the only natural precursor of cortisol.
2. The 17-hydroxylation mechanism may be more susceptible to *in vitro* conditions, than are the other hydroxylating mechanisms.

3. The rates of release (transport?) of cortisol and corticosterone from the synthetic site (cell) to the blood may be of different orders.

Eichhorn and Hechter's (20) recent report on experiments in which progesterone was incubated with bovine adrenal tissue, indicate a greater synthesis of corticosterone as compared to cortisol.

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JAUNDICE DURING ADMINISTRATION OF METHYLESTRENOLONE

To the Editor:

Reversible, intrahepatic, obstructive jaundice with microscopic cholangiolitis and intralobular cholestasis has been reported in some 20 patients receiving methyltestosterone (1, 2). Since there is considerable current interest in the 19-nor steroids (3, 4, 5) we wish to report the occurrence of the same phenomenon in 2 of 10 patients during therapeutic trials of sublingual tablets of 17-methyl-19-nortestosterone (methylestrenolone or 17MNT). The outline of the study has been reported elsewhere (6). One of the patients was a 62-year-old man with metastatic prostatic carcinoma and angina treated by inducing myxedema with I^{131}. Icterus developed after four weeks of treatment with methylestrenolone in a dosage of 25 mg. daily followed by fourteen weeks of treatment with 6 mg. daily. Jaundice was attributed to metastatic involvement of the liver and/or congestive failure, but it subsided during continuation of therapy. However, at the time of death four weeks later, the histologic picture was that of resolving methyltestosterone jaundice, and serum bilirubin concentration was still slightly elevated (1.2 mg. per 100 ml.). The other patient, a 46-year-old man with a history of previous alcoholism and myocardial infarct, received methylestrenolone in a dosage of 300 mg. daily for six weeks. Upon omission of therapy, he was found to have deeply icteric serum. Two days later he returned to the hospital, deeply icteric. The laboratory findings were indicative of acute biliary obstruction. Icterus and gastro-intestinal distress subsided rapidly with symptomatic and supportive therapy. Sub-

sequent hepatic biopsy revealed histologic changes similar to those seen following methyltestosterone jaundice.

These findings suggest that methylestrenolone has the same potential for disturbing hepatic function as has methyltestosterone, and that others studying the compound may observe this complication. However it is probable that the occurrence of jaundice in 20 per cent of our small series of cases is coincidental, as other physicians have employed it in similar doses in larger numbers of individuals without observing jaundice (3).

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The Endocrine Society

THE 1958 ANNUAL MEETING

The Fortieth Annual Meeting of The Endocrine Society will be held in the St. Francis Hotel, San Francisco, California, Thursday, Friday and Saturday, *June 19, 20, and 21, 1958.*

The Committee on Local Arrangements—Dr. Roberto Escamilla, Chairman; Dr. Donald E. Bernstein, Dr. Peter H. Forsham, Dr. Minnie B. Goldberg, Dr. Gilbert S. Gordan, Dr. Francis S. Greenspan, Dr. C. H. Li, as members of the committee.

All Scientific Sessions will be held from 9:00 A.M. to 5:00 P.M. daily in the Colonial Ballroom, and in addition there will be simultaneous afternoon sessions in the Italian Room. The annual dinner is scheduled for Friday, June 20 at 7:30 P.M., preceded by cocktails at 6:30 P.M.

All reservations for rooms must be made through the AMA Housing Bureau, Room 300, 61 Grove Street, San Francisco 2, California, after January 1, 1958. No reservations will be accepted before that date. If possible, official application forms as published in the Journal of the American Medical Association should be used. Arrival and departure dates must be stated. All requests must be accompanied by check or money order (not cash) made payable to "The AMA Housing Bureau" in the amount of $10.00 per room, which will be credited to your hotel account. Be sure to state your first, second and third choice of hotels. It is important to make reservations immediately in order to avoid disappointment.

Final program, membership card and advance registration forms will be sent on May 15, 1958 to members whose current dues have been paid.

Those wishing to present papers, which will be strictly limited to ten minutes, should send four copies of the title and abstract to the Vice-President, Dr. Rulon W. Rawson, 444 E. 68th St., New York 21, N. Y., not later than *February 1, 1958. It is imperative that the abstracts be informative and complete with results and conclusions—not a statement that those will be presented at the meeting*—in order that they may be of reference value and suitable for printing in the program. The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. Reference, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.

2. The title heading must be arranged as follows:

   Line 1. Title, not to exceed fifteen words;

   Line 2. Author/s. The name of each nonmember-author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of nonmembers who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be written after his name.

   Line 3. Institution of origin and city in which institution is located.

3. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three copies.
4. Abstracts should be letter perfect, since there will be no opportunity for proofreading by the authors.

THE 1958 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada. Dr. Carl R. Moore was the recipient in 1955, Dr. Frederick L. Hisaw in 1956, and Dr. Joseph C. Aub in 1957.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—

Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

The American Goiter Association

THE 1958 ANNUAL MEETING

The next Annual Meeting of The American Goiter Association will be held in the St. Francis Hotel, San Francisco, California, on *June 17, 18 and 19, 1958.*

THE VAN METER PRIZE AWARD FOR 1958

The American Goiter Association again offers the Van Meter Prize Award of $300.00 and two honorable mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The award will be made at the annual meeting of the Association, which will be held in the St. Francis Hotel, San Francisco, California, June 17, 18 and 19, 1958, provided essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations, should not exceed 3,000 words in length and must be presented in English. Duplicate typewritten copies, double spaced, should be sent to the Secretary, Dr. John C. McClintock, 149½ Washington Avenue, Albany 10, N. Y., *not later than February 1, 1958.* The committee who will review the manuscripts is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for the presentation of the winning essay by the author if it is possible for him to attend.

AMERICAN CANCER SOCIETY CLINICAL FELLOWSHIPS

The American Cancer Society has announced that Clinical Fellowships at the senior resident level for the academic year 1959–60 may be applied for by institutions accredited by the Council on Medical Education and Hospitals of the American Medical Association to give training in the following specialties and sub-specialties, with emphasis on the diagnosis and treatment of cancer: internal medicine, malignant diseases, neurological surgery, obstetrics-gynecology, orthopedic surgery, otolaryngology, pathology, public health, radiology, surgery, and urology.

Institutions will be notified of awards granted in June 1958. Individual candidates should apply directly to an institution, or the American Cancer Society, for information concerning fellowships. The annual stipend, tax exempt, is $3,600.

Application forms are available from the Director of Professional Education, American Cancer Society, Inc., 521 West 57th Street, New York 19, N. Y.

February 15, 1958 is the deadline for institutions submitting applications for the 1959–60 clinical fellowships.

ANNOUNCEMENT OF NEW QUARTERLY JOURNAL

Perspectives in Biology and Medicine, a new quarterly journal dedicated to a multidisciplined approach to the problems of biology and medicine, is announced by the Division of Biological Sciences and the Press of the University of Chicago.

Perspectives' editors, Dr. Dwight J. Ingle and Dr. S. O. Waife, among many other eminent scientists, have long felt the need of a journal oriented toward man and his diseases but with appreciation of the fact that the roots of medical theory reach into all fields of biology and all processes of life—a journal to communicate and stimulate original thought in the biological and medical sciences and to be inspirational to young men and women to think beyond the confines of specialization.

Perspectives will present new hypotheses and concepts representing informed thinking; interpretative, selective essays which take stock of the implications of recent and current research and indicate strategy for the future; and, because perspective and inspiration can also be gained by looking to the past, reviews by great men in science and medicine of their formative years and their philosophy of research.

Subscriptions, at $6.00 per year, may be sent to The University of Chicago Press, 5750 Ellis Avenue, Chicago 37, Illinois.